COMPLEMENT RECEPTOR 2 TARGETED COMPLEMENT MODULATORS

This application claims benefit of U.S. Provisional Application No. 60/426,676, filed on November 15, 2002, which is incorportated herein by reference in its entirety.

I. BACKGROUND OF THE INVENTION

1. Complement is the collective term for a series of blood proteins and is a major effector mechanism of the immune system. Complement activation and its deposition on target structures can lead to direct complement-mediated cell lysis, or can lead indirectly to cell or tissue destruction due to the generation of powerful modulators of inflammation and the recruitment and activation of immune effector cells. Complement activation products that mediate tissue injury are generated at various points in the complement pathway. Inappropriate complement activation on host tissue plays an important role in the pathology of many autoimmune and inflammatory diseases, and is also responsible for many disease states associated with bioincompatibility, e.g. post-cardiopulmonary inflammation and transplant rejection. Complement inhibition represents a potential therapeutic modality for the treatment of such immune-mediated diseases and disease states. Complement inhibitory proteins that systemically inhibit complement have been shown to be effective in various animal models of disease (and in a few clinical trials), but complement inhibitors that target a site of disease and complement activation offer significant potential advantages with regard to safety and efficacy.

2. In healthy individuals, complement deposition on host cell membranes is prevented by complement inhibitory proteins expressed at the cell surface. These complement inhibitory proteins are also expressed on the surface of tumor cells, often at increased levels, and are considered to be an important contributing factor to the resistance of tumor cells to monoclonal antibody-mediated immunotherapy (monoclonal antibodies that target to tumor cells and activate complement).

3. The complement system comprises a collection of about 30 proteins and is one of the major effector mechanisms of the immune system. The complement cascade is activated principally via either the classical (usually antibody-dependent) or

alternative (usually antibody-idependent) pathways. Activation via either pathway leads to the generation of C3 convertase, which is the central enzymatic complex of the cascade. C3 convertase cleaves serum C3 into C3a and C3b, the latter of which binds covalently to the site of activation and leads to the further generation of C3 convertase (amplification loop). The activation product C3b (and also C4b generated only via the classical pathway) and its breakdown products are important opsonins and are involved in promoting cell-mediated lysis of target cells (by phagocytes and NK cells) as well as immune complex transport and solubilization. C3/C4 activation products and their receptors on various cells of the immune system are also important in modulating the cellular immune response. C3 convertases participate in the formation of C5 convertase, a complex that cleaves C5 to yield C5a and C5b. C5a has powerful proinflammatory and chemotactic properties and can recruit and activate immune effector cells. Formation of C5b initiates the terminal complement pathway resulting in the sequential assembly of complement proteins C6, C7, C8 and (C9)n to form the membrane attack complex (MAC or C5b-9). Formation of MAC in a target cell membrane can result in direct cell lysis, but can also cause cell activation and the expression/release of various inflammatory modulators.

4. There are two broad classes of membrane complement inhibitor; inhibitors of the complement activation pathway (inhibit C3 convertase formation), and inhibitors of the terminal complement pathway (inhibit MAC formation). Membrane inhibitors of complement activation include complement receptor 1 (CR1), decay-accelerating factor (DAF) and membrane cofactor protein (MCP). They all have a protein structure that consists of varying numbers of repeating units of about 60-70 amino acids termed short consensus repeats (SCR) that are a common feature of C3/C4 binding proteins. Rodent homologues of human complement activation inhibitors have been identified. The rodent protein Crry is a widely distributed inhibitor of complement activation that functions similar to both DAF and MCP. Rodents also express DAF and MCP, although Crry appears to be functionally the most important regulator of complement activation in rodents. Although there is no homolog of Crry found in humans, the study of Crry and its use in animal models is clinically relevant.

5. Control of the terminal complement pathway and MAC formation in host cell membranes occurs principally through the activity of CD59, a widely distributed 20kD glycoprotein attached to plasma membranes by a glucosylphosphatidylinositol (GPI) anchor. CD59 binds to C8 and C9 in the assembling MAC and prevents membrane insertion.

6. Various types of complement inhibitory proteins are currently under investigation for therapy of inflammatory disease and disease states associated with bioincompatibility. Two of the best therapeutically characterized inhibitors of human complement are a soluble form of complement receptor 1 (sCR1) and an anti-C5 monoclonal antibody. These systemically active inhibitory proteins have shown efficacy in various animal models of disease and more recently in clinical trials (1-5, 6:#1037). Anti-C5 mAb inhibits the generation of C5a and the MAC, whereas sCR1 is an inhibitor of complement activation and also inhibits the generation of C3 activation products. Soluble forms of human decay accelerating factor (DAF) and membrane cofactor protein (MCP), membrane inhibitors of complement activation, have also been shown to be protective an animal models of inflammation and bioincompatability (7-11). CD59 is a membrane inhibitor of complement that blocks assembly of the MAC, but does not effect generation of complement opsonins or C3a and C5a. Soluble forms of CD59 have been produced, but its low functional activity in vitro, particularly in the presence of serum, indicates that sCD59 will have little or no therapeutic efficacy (12-15).

7. Targeting complement inhibitors to sites of complement activation and disease is likely to improve their efficacy. Since complement plays an important role in host defense and immune complex catabolism, targeted complement inhibitors can also reduce potentially serious side effects, particularly with long term complement inhibition. Recently, a modified form of sCR1 decorated with sialyl Lewis x (sLex) was prepared and shown to bind to endothelial cells expressing P and E selectin. sCR1sLex was shown to be a more potent therapeutic than sCR1 in rodent models of inflammatory disease (16, 17). In in vitro feasibility studies, antibody-DAF (18) and antibody-CD59 (19) fusion proteins were shown to be more effective at protecting targeted cells than

untargeted cells from complement. Non-specific membrane targeting of recombinant complement inhibitors has also been achieved by coupling inhibitors to membrane-inserting peptides (20, 21).

8. C3 activation fragments are abundant complement opsonins found at a site of complement activation, and they serve as ligands for various C3 receptors. One such receptor, complement receptor 2 (CR2), a transmembrane protein, plays an important role in humoral immunity by way of its expression predominantly on mature B cells and follicular dendritic cells (22, 23). CR2 is a member of the C3 binding protein family and consists of 15-16 short consensus repeat (SCR) domains, structural units that are characteristic of these proteins, with the C3 binding site being contained in the two N-terminal SCRs (24, 25). CR2 is not an inhibitor of complement and it does not bind C3b, unlike the inhibitors of complement activation (DAF, MCP, CR1 and Crry). Natural ligands for CR2 are iC3b, C3dg and C3d, cell-bound breakdown fragments of C3b that bind to the two N-terminal SCR domains of CR2 (26, 27). Cleavage of C3 results initially in the generation and deposition of C3b on the activating cell surface. The C3b fragment is involved in the generation of enzymatic complexes that amplify the complement cascade. On a cell surface, C3b is rapidly converted to inactive iC3b, particularly when deposited on a host surface containing regulators of complement activation (ie. most host tissue). Even in absence of membrane bound complement regulators, substantial levels of iC3b are formed. iC3b is subsequently digested to the membrane bound fragments C3dg and then C3d by serum proteases, but this process is relatively slow (28, 29). Thus, the C3 ligands for CR2 are relatively long lived once they are generated and will be present in high concentrations at sites of complement activation.

II. SUMMARY OF THE INVENTION

9. In accordance with the purposes of this invention, as embodied and broadly described herein, this invention, in one aspect, relates to CR2 targeted modulators of complement activity.

10. Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or can be

learned by practice of the invention. The advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

III. BRIEF DESCRIPTION OF THE DRAWINGS

11. The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention and together with the description, serve to explain the principles of the invention.

12. Figure 1 shows a diagram of examples of CR2-complement inhibitor fusion proteins.

13. Figure 2 shows SDS-PAGE and Western blot analysis of purified recombinant fusion proteins and soluble complement inhibitors. Gels (10% acrylamide) were stained with coomasie blue. Western blots were developed using antibodies to complement inhibitors as the primary antibody.

14. Figure 3 shows binding of recombinant fusion proteins to C3-opsonized CHO cells. Antibody sensitized CHO cells were incubated in C6-deficient serum, washed and incubated with soluble complement inhibitor (black trace), or fusion protein with CR2 at N-terminus (light gray trace) or C-terminus (dark gray trace) at 20μg/ml. Cell binding of recombinant proteins was detected by flow cytometry using anti-DAF or anti-CD59 mAbs. Incubation of CHO cells with PBS instead of complement inhibitor gave similar fluorescence profile as sDAF and sCD59. Representative of 3 separate experiments.

15. Figure 4 shows analysis of the interaction between CR2 fusion proteins and C3d by surface plasmon resonance. Solid lines indicate different concentrations of CR2 fusion proteins as indicated in Figure___[Looks like a fig. no. is missing.]. Broken lines show curves fitting to a 1:1 Langmuir binding model.

16. Figure 5 shows inhibition of complement mediated lysis by recombinant sDAF and DAF fusion proteins. Antibody sensitized CHO cells (panel a) or sheep

erythrocytes (panel b) were incubated with recombinant protein and 10% human serum (CHO cells) or 0.33% human serum (erythrocytes). These concentrations resulted in approximately 90% lysis of unprotected cells. Lysis was determined after 45 min. incubation at 37°C. Background lysis determined by incubating cells in heat inactivated serum was less than 5% and was subtracted. Mean +/- SD, n = 4.

17. Figure 6 shows inhibition of complement mediated lysis by recombinant sCD59 and CD59 fusion proteins. Antibody sensitized CHO cells (panel a) or sheep erythrocytes (panel b) were incubated with recombinant protein and 10% human serum (CHO cells) or 0.33% human serum (erythrocytes). These concentrations resulted in approximately 90% lysis of unprotected cells. Lysis was determined after 45 min. incubation at 37°C. Background lysis determined by incubating cells in heat inactivated serum was less than 5% and was subtracted. Mean +/- SD, n = 4.

18. Figure 7 shows the effect of recombinant fusion proteins on U937 cell adhesion. Sheep erythrocytes were sensitized with IgM antibody and incubated in C6-deficient serum. C3 opsonized erythrocytes were coincubated with U937 cells in the presence of 500 nM recombinant fusion protein or PBS. Following incubation, the average number of U937 cells bound per erythrocyte was determined my microscopy. Mean +/- SD, n = 3.

19. Figure 8 shows the nucleotide and predicted amino acid sequence of mature human CR2-DAF. Amino acids underlined represent linking sequences between CR2 and DAF.

20. Figure 9 shows the nucleotide and predicted amino acid sequence of mature human CR2-CD59. Amino acids underlined represent linking sequences between CR2 and CD59.

21. Figure 10 shows the nucleotide and predicted amino acid sequence of mature human DAF-CR2. Amino acids underlined represent linking sequences between DAF and CR2.

22. Figure 11 shows the nucleotide and predicted amino acid sequence of mature human CD59-CR2. Amino acids underlined represent linking sequences between CD59 and CR2.

23. Figure 12 shows targeting of CR2 containing fusion proteins to C3-coated CHO cells. C3 ligand was generated on CHO cells by incubation of cells in 10% anti-CHO antiserum and 10% C6-depleted human serum (to prevent formation of membrane attack complex and cell lysis). Cells were washed and incubated with fusion protein (20 ug/ml, 4°C, 30 min). Binding was detected by flow cytometric analysis using antibodies against appropriate complement inhibitor (DAF or CD59). Black line: control (no fusion protein); Light gray: CR2 at C-terminus; Dark gray: CR2 at N-terminus.

24. Figure 13 shows analysis of CR2-DAF binding to C3dg by surface plasmon resonance.

25. Figure 14 shows analysis of CR2-CD59 binding to C3dg by surface plasmon resonance.

26. Figure 15 shows analysis of DAF-CR2 binding to C3dg by surface plasmon resonance.

27. Figure 16 shows analysis of CD59-CR2 binding to C3dg by surface plasmon resonance.

28. Figure 17 shows the effect of targeted and untargeted DAF on complement-mediated lysis of CHO cells. CHO cells were sensitized to complement with anti-CHO antisera (10% concentration, 4°C, 30 min) and subsequently incubated with 10% normal human serum (NHS) (37°C, 60 min) in the presence of varying concentrations of complement inhibitory proteins. Cell lysis was then determined by trypan blue exclusion assay. Representative experiment showing mean +/- SD (n=3). Three separate experiments using different fusion protein preparations performed.

29. Figure 18 shows the effect of targeted and untargeted CD59 on complement-mediated lysis of CHO cells. Assay performed as described in legend to Figure 17. Representative experiment showing mean +/- SD (n=3). Three separate experiments using different fusion protein preparations performed.

30. Figure 19 shows the effect of targeted and untargeted DAF on complement-mediated hemolysis. Sheep erythrocytes (E) were sensitized with anti-sheep E antibody and subsequently incubated with a 1/300 dilution of NHS (37°C, 60 min) in the presence of varying concentrations of complement inhibitory proteins. Cell lysis was

determined by measuring released hemoglobin (absorbance at 412 nm). Representative experiment showing mean +/- SD (n=3). Two separate experiments using different fusion protein preparations performed.

31. Figure 20 shows the effect of targeted and untargeted CD59 on complement-mediated hemolysis. Assay performed as described in legend to Figure 19. Representative experiment showing mean +/- SD (n=3). Two separate experiments using different fusion protein preparations performed.

32. Figure 21 shows the nucleotide and predicted amino acid sequence of mature human CR2-human IgG1 Fc. Amino acids underlined represent linking sequences between CR2 and Fc region. Expression plasmid contains genomic Fc region (hinge-intron-CH2-intron-CH3).

33. Figure 22 shows SDS-PAGE analysis of CR2-Fc fusion protein. Purified CR2-Fc was run under nonreducing (lane 1) or reducing (lane 2) conditions. Gel stained by coomassie blue. (for MW of markers in lane 3, see Figure 2).

34. Figure 23 shows targeting of CR2-Fc to C3-coated CHO cells. C3 ligand was generated as described (legend to Figure 12). Cells were washed and incubated with CR2-Fc (20 ug/ml, 4^oC, 30 min). Binding was detected by flow cytometric analysis using antibodies against human Fc conjugated to FITC. Upper panel shows results from incubation of CR2-Fc with C3-coated CHO cells, and lower panel shows results from incubation of CR2-Fc with control CHO cells.

35. Figure 24 shows surface plasmon resonance sensorgram showing binding of CR2-Fc to C3d ligand immobilized on chip.

36. Figure 25 shows the biodistribution of ^{125}I-CR2-DAF and ^{125}I-sDAF in 34 week old NZB/W F1 mice. Radiolabeled proteins were injected into the tail vein and biodistribution of radiolabel determined after 24 hr. Each protein was injected into 2 mice.

37. Figure 26 shows imaging of CR2-DAF bound to glomeruli of 24-week-old MRL/lpr mice. Glomerular binding of CD2-DAF (a) and sDAF (b) was analyzed 24 hours after tail-vein injection of each protein. The figure shows immunofluorescence staining of kidney sections.

38. Figure 27 shows the single chain antibody CD59-Crry construct. The figure shows the construct comprises a variable light chain (VL) and a variable heavy chain (VH) from K9/9 mAb. The construct was prepared in the yeast expression vector pPICZalph (Invitrogen).

39. Figure 28 shows the biodistribution of complement inhibitors and K9/9 single chain Ab in rats. Iodinated recombinant proteins administered 4 days after PAN treatment and radioactivity in organs measured 48 hr later.

40. Figure 29 shows Creatinine clearance in rats treated with PAN and receiving indicated therapy (n = 4, +/- SD).

41. Figure 30 shows PAS stained renal cortex. Figure 30A shows No PAN control, Figure 30B: PAN with PBS treatment, Figure 30C: PAN with targeted K9/9 Crry treatment, and Figure 30D: PAN with sCrry treatment.

42. Figure 31 shows complement inhibitory activity in serum after administration of recombinant proteins. Measured by lysis of sensitized sheep erythrocytes. Percent inhibitory activity shown relative to serum from control rats.

IV. DETAILED DESCRIPTION

43. The present invention can be understood more readily by reference to the following detailed description of preferred embodiments of the invention and the Examples included therein and to the Figures and their previous and following description.

44. Before the present compounds, compositions, articles, devices, and/or methods are disclosed and described, it is to be understood that this invention is not limited to specific synthetic methods, specific recombinant biotechnology methods unless otherwise specified, or to particular reagents unless otherwise specified, as such can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

A. Definitions

45. As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

46. Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

47. In this specification and in the claims which follow, reference will be made to a number of terms which shall be defined to have the following meanings:

48. "Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

49. "Treatment" or "treating" means to administer a composition to a subject with a condition, wherein the condition can be any pathogenic disease, autoimmune disease, cancer or inflammatory condition. The effect of the administration of the composition to the subject can have the effect of but is not limited to reducing the symptoms of the condition, a reduction in the severity of the condition, or the complete ablation of the condition.

50. Herein, "inhibition" or "inhibits" means to reduce activity. It is understood that inhibition can mean a slight reduction in activity to the complete ablation of all activity. An "inhibitor" can be anything that reduces activity.

51. Herein, "activation"or "activates" means to increase activity. It is understood that activation can mean an increase in existing activity as well as the induction of new activity. An "activator" can be anything that increases activity.

B. Complement Inhibiting and Activating Constructs

52. Discosed are compositions comprising a construct, wherein the construct comprises CR2 and a modulator of complement activity.

53. CR2 consists of an extracellular portion consisting of 15 or 16 repeating units known as short consensus repeats (SCRs). Amino acids 1-20 comprise the leader peptide, amino acids 23-82 comprise SCR1, amino acids 91-146 comprise SCR2, amino acids 154-210 comprise SCR3, amino acids 215-271 comprise SCR4. The active site (C3dg binding site) is located in SCR 1-2 (the first 2 N-terminal SCRs). SCR units are separated by short sequences of variable length that serve as spacers. It is understood that any number of SCRs containing the active site can be used. In one embodiment, the construct contains the 4 N-terminal SCR units. In another embodiment, the construct includes the first two N-terminal SCRs. In another embodiment the construct includes the first three N-terminal SCRs.

54. It is understood that species and strain variation exist for the disclosed peptides, polypeptides, proteins, protein fragments and compositions. Specifically disclosed are all species and strain variations for the disclosed peptides, polypeptides, proteins, protein fragments and compositions.

55. Also disclosed are compositions, wherein the construct is a fusion protein

56. Herein a "fusion protein" means two or more components comprising peptides, polypeptides, or proteins operably linked. CR2 can be linked to complement inhibitors or activators by an amino acid linking sequence. Examples of linkers are well known in the art. Examples of linkers can include but are not limited to $(Gly_4Ser)_3$ (G4S), $(Gly_3Ser)_4$ (G3S), $SerGly_4$, and $SerGly_4SerGly_4$. Linking sequences can also consist of "natural" linking sequences found between SCR units within human (or mouse) proteins, for example VSVFPLE, the linking sequence between SCR 2 and 3 of human CR2. Fusion proteins can also be constructed without linking sequences.

57. Also disclosed are compositions of the invention, wherein the fusion protein inhibits complement.

58. Also disclosed are compositions of the invention, wherein the modulator of complement activity comprises a complement inhibitor.

59. Also disclosed are compositions of the invention; for example, wherein the complement inhibitor is decay accelerating factor (DAF) SEQ ID NO: 1 (nucleotide) and SEQ ID NO: 2 (amino acid). For example, the DAF can be soluble human DAF comprising the four SCR domains without glycophosphatidyl anchor and serine-threonine rich region. The DAF can also be soluble human DAF comprising the four SCR domains and the serine-threonine rich region but without glycophosphatidyl anchor.

60. The DAF extracellular region consists of 4 SCR units at N-terminus followed by serine/threonine rich region. Amino acids 1-34 comprise the leader peptide, amino acids 35-95 comprise SCR1, amino acids 97-159 comprise SCR2, amino acids 162-221 comprise SCR3, amino acids 224-284 comprise SCR4, and amino acids 287-356 comprise the S/T region. In one embodiment of the invention, the composition of the invention comprises all 4 SCR units. In another emobodiement of the invention, the composition comprises SCR2-4 of DAF.

61. Disclosed are compositions of the invention, wherein the complement inhibitor comprises a fusion protein between CD59 and another complement inhibitor selected from the group consisting of DAF, MCP, Crry and CR1. Also disclosed are compositions of the invention, wherein the complement inhibitor is a fusion protein of two or more complement inhibitors.

62. Also disclosed are compositions of the invention, wherein the fusion protein comprises CR2-DAF (SEQ ID NO: 6). Also disclosed are compositions of the invention wherein the fusion protein is encoded by a nucleotide comprising SEQ ID NO: 5.

63. Also disclosed are compositions of the invention, wherein the fusion protein comprises DAF-CR2 (SEQ ID NO: 10). Also disclosed are compositions of the invention wherein the fusion protein is encoded by a nucleotide comprising SEQ ID NO: 9.

64. Also disclosed are compositions of the invention, wherein the complement inhibitor is human CD59 (SEQ ID NO: 3 (nucleotide) and SEQ ID NO: 4 (amino

acid)). The human CD59 can be soluble human CD59 comprising the mature protein without glycophosphatidyl anchor.

65. Also disclosed are compositions of the invention, wherein the fusion protein comprises CR2-human CD59 (SEQ ID NO: 8). Also disclosed are compositions of the invention wherein the fusion protein is encoded by a nucleotide comprising SEQ ID NO: 7.

66. Also disclosed are compositions of the invention, wherein the fusion protein comprises human CD59-CR2 (SEQ ID NO: 12). Also disclosed are compositions of the invention wherein the fusion protein is encoded by a nucleotide comprising SEQ ID NO: 10.

67. Also disclosed are compositions of the invention wherein the complement inhibitor is an antibody to C5. Also disclosed are compositions of the invention, wherein the fusion protein comprises CR2-anti-C5 antibody.

68. Also disclosed are compositions of the invention, wherein the complement inhibitor is CR1 (SEQ ID NO: 13 (nucleotide) and SEQ ID NO: 14 (amino acid)). The extracellular region of CR1 can comprise 30 SCR units. It is an embodiment of the invention that the composition can comprise the entire extracellular region of CR1. In another embodiment of the invention, the composition comprises [the] one active site[s]. of CR1. The active sites of CR1 are amino acids 1– 46 which comprise the leader peptide, amino acids 47-300 which comprise SCR1-4 (C4b binding site, lower affinity for C3b), amino acids 497-750 which comprise SCR8-11 (C3b binding site, lower affinity for C4b), amino acids 947-1200 which comprise SCR15-18 (C3b binding site, lower affinity for C4b), and amino acids 1400-1851 which comprise the C1q binding site. In an additional embodiment of the invention, the composition of the invention can comprise any [one or] combination or all of the active sites of CR1.

69. Also discosed are are compositions of the ivention, wherein the complement inhibitor comprises the active sites of CR1, and wherein [the] one active site[s] further comprise a leader peptide comprising amino acids 6-46, amino acids 47-300 which comprise SCR1-4 (C4b binding site, lower affinity for C3b), amino acids 497-750 which comprise SCR8-11 (C3b binding site, lower affinity for C4b), amino acids 947-

1200 which comprise SCR15-18 (C3b binding site, lower affinity for C4b), and amino acids 1400-1851 which comprise the C1q binding site. In an additional embodiment of the invention, the composition of the invention can comprise any [one or] combination or all of the active sites of CR1.

70. Also disclosed are compositions of the invention, wherein the complement inhibitor is MCP (SEQ ID NO: 15 (nucleotide) and SEQ ID NO: 16 (amino acid)). The extracellular region consists of 4 SCR units followed by ser/thr region. Amino acids 1-34 comprise the leader peptide, amino acids 35-95 comprise SCR1, amino acids 96-158 comprise SCR2, amino acids, 159-224 comprise SCR3, amino acids 225-285 comprise SCR4, and amino acids 286-314 comprise the S/T region

71. Also disclosed are compositions of the invention, wherein the complement inhibitor is Crry (SEQ ID NO: 17). The Crry can be soluble mouse Crry comprising the 5 N-terminal SCR domains without transmembrane region.

72. Also disclosed are compositions of the invention, wherein the complement inhibitor is murine CD59. The murine CD59 can be soluble murine CD59 comprising the mature protein without glycophosphatidyl anchor.

73. Disclosed are compositions of the invention, wherein the fusion protein activates complement.

74. Thus, disclosed are compositions of the invention, wherein the modulator of complement activity comprises a complement activator.

75. Disclosed are compositions of the invention, wherein the complement activator is human IgG1 Fc(SEQ ID NO: 18).

76. Also disclosed are compositions of the invention, wherein the complement activator comprises CR2-human IgG1 Fc (SEQ ID NO: 20). Also disclosed are compositions of the invention wherein the fusion protein is encoded by a nucleotide comprising SEQ ID NO: 21.

77. Disclosed are compositions of the invention, wherein the fusion protein is human IgM (SEQ ID NO: 19).

78. Also disclosed are compositions of the invention, wherein the fusion protein comprises CR2-human IgM Fc.

79. Disclosed are compositions of the invention, wherein the complement activator is mouse IgG3 (SEQ ID NO: 22).

80. Also disclosed are compositions of the invention, wherein the fusion protein comprises CR2-murine IgG3 Fc.

81. Also disclosed are compositions of the invention, wherein the fusion protein comprises CR2-murine IgM Fc.

82. It is specifically contemplated that complement activator can also increase antibody-dependent cell-mediated cytotoxicity (ADCC) via the Fc portion of the composition. ADCC is the destruction of a target cell by a natural killer (NK) cell via recognition of and contact with an Fc region and an Fc receptor on the NK cell. This can be in the form of FcγRIII recognition of IgG1 Fc or IgG3 Fc. Following the contact of the Fc receptor with the Fc, the NK cell lysis the target cell via the use of perforin and granzyme. This mechanism can be important in controlling tumor growth

83. Disclosed are compositions of the invention, wherein the CR2-Fc fusion protein is not immunogenic. It is understood that a composition that is not immunogenic (ie. does not elicit an immune response) is less likely to be attacked and inactivated by the subjects own immune response. The anticipated lack of CR2-Fc immunogenicity is a potential advantage over anti-C3d antibodies, even if antibodies are humanized. It is an embodiment of the invention that the Fc region fused to CR2 can be from any human or mouse IgG isotype, human or mouse IgM, or any human or mouse IgG isotype containing a mu-tailpiece. The mu-tailpiece is an 18 amino acid C-terminal region from IgM that, when added C-terminal to IgG Fc sequences, results in the generation of polymeric forms of IgG (similar to IgM) that efficiently activate complement and have enhanced affinity for Fc receptors. The fusion can occur at the hinge region of the Fc portion of the composition.

84. CR2 fusion proteins containing either IgM or IgG Fc regions with a mu-tailpiece can have advantages over CR2-IgG Fc fusion proteins. IgM or IgG-mu Fc regions will result in polymeric fusion proteins with up to 6 Fcs and 12 CR2 sites. These constructs can have enhanced avidity for C3 ligand and enhanced effector function (complement activation and Fc receptor binding).

85. Also disclosed are compositions of the invention, wherein the complement activator is CVF (SEQ ID NO: 23 (nucleotide) and SEQ ID NO: 24 (amino acid)).

86. In one embodiment of the invention, CVF can be coupled to soluble CR2. It is understood that CVF binds factor B and activates the alternative pathway of complement by forming CVFBb, a C3/C5 convertase that is not inactivated by complement inhibitory proteins. The half life of CVFBb is about 7 hr. compared to about 1 min. for the physiological alternative pathway convertase, C3bBb.

87. It is an embodiment of the invention that CVF can be chemically coupled to soluble CR2.

88. Disclosed are compositions of the invention, wherein the construct is in a vector.

89. Disclosed are cells comprising the vector of the invention.

90. Also disclosed are compositions, wherein the construct is an immunoconjugate. Herein "immunoconjugate" means two or more components comprising peptides, polypeptides, or proteins operably linked by a chemical cross-linker. Linking of the components of the immunoconjugate can occur on reactive groups located on the component. Reactive groups that can be targeted using a cross-linker include primary amines, sulfhydryls, carbonyls, carbohydrates and carboxylic acids, or active groups can be added to proteins. Examples of chemical linkers are well known in the art and can include but are not limited to bismaleimidohexane, m-maleimidobenzoyl-N-hydroxysuccinimide ester, NHS-Esters-Maleimide Crosslinkers such as MBS, Sulfo-MBS, SMPB, Sulfo-SMPB, GMBS, Sulfo-GMBS, EMCS, Sulfo-EMCS; Imidoester Cross-linkers such as DMA, DMP, DMS, DTBP; EDC [1-Ethyl-3-(3-Dimethylaminopropyl)carbodiimide Hydrochloride], [2-(4-Hydroxyphenyl)ethyl]-4-N-maleimidomethyl)-cyclohexane-1-carboxamide, DTME: Dithio-bis-maleimidoethane, DMA (Dimethyl adipimidate•2 HCl), DMP (Dimethyl pimelimidate•2 HCl), DMS (Dimethyl suberimidate•2 HCl), DTBP (Dimethyl 3,3'-dithiobispropionimidate•2HCl), MBS, (*m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester), Sulfo-MBS (*m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester), Sulfo-SMPB (Sulfosuccinimidyl 4-[*p*-maleimidophenyl]butyrate(, GMBS (*N*-[•-

maleimidobutyryloxy]succinimide ester), EMCS (*N*-[•-maleimidocaproyloxy]succinimide ester), and Sulfo-EMCS (*N*-[•-maleimidocaproyloxy]sulfosuccinimide ester).

C. Methods of using the compositions

91. Various types of complement inhibitory proteins are currently under investigation for therapy of inflammatory disease and disease states associated with bioincompatibility. Two of the best therapeutically characterized inhibitors of human complement are a soluble form of complement receptor 1 (sCR1) and an anti-C5 monoclonal antibody. These systemically active inhibitory proteins have shown efficacy in various animal models of disease and more recently in clinical trials (1-5, 6:#1037 which are incorporated herein by reference regarding teachings on *in vivo* efficacy and clinical results).

92. Disclosed are methods of treating a condition affected by complement in a subject comprising administering to the subject the composition of the invention. It is understood that administration of the composition to the subject can have the effect of, but is not limited to, reducing the symptoms of the condition, a reduction in the severity of the condition, or the complete ablation of the condition.

1. Methods of using the compositions to inhibit complement

93. Disclosed are methods of treating a condition affected by complement in a subject comprising administering to the subject the composition of the invention, wherein the composition will inhibit complement activity. It is understood that the effect of the administration of the composition to the subject can have the effect of but is not limited to reducing the symptoms of the condition, a reduction in the severity of the condition, or the complete ablation of the condition.

94. Disclosed are methods of reducing complement-mediated damage comprising administering to a subject the composition of the invention, which inhibits complement.

95. Disclosed are methods of the invention, wherein the condition treated is an inflammatory condition. Also disclosed are methods of the invention, wherein the

inflammatory condition can be selected from the group consisting of asthma, systemic lupus erythematosus, rheumatoid arthritis, reactive arthritis, spondylarthritis, systemic vasculitis, insulin dependent diabetes mellitus, multiple sclerosis, experimental allergic encephalomyelitis, Sjögren's syndrome, graft versus host disease, inflammatory bowel disease including Crohn's disease, ulcerative colitis, ischemia reperfusion injury, myocardial infarction, alzheimer's disease, transplant rejection (allogeneic and xenogeneic), thermal trauma, any immune complex-induced inflammation, glomerulonephritis, myasthenia gravis, cerebral lupus, Guillain-Barre syndrome, vasculitis , systemic sclerosis, anaphlaxis, catheter reactions, atheroma, infertility, thyroiditis, ARDS, post-bypass syndrome, hemodialysis, juvenile rheumatoid, Behcets syndrome, hemolytic anemia, pemphigus, bullous pemphigoid, stroke, atherosclerosis, and scleroderma.

96. Also disclosed are methods of the invention, wherein the condition is a viral infection. Also disclosed are methods of the invention, wherein the viral infection can be selected from the list of viruses consisting of Influenza virus A, Influenza virus B, Respiratory syncytial virus, Dengue virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Hantavirus.

97. Disclosed are methods of the invention, wherein the condition is an inflammatory respose to a viral vector. The viral vector can be selected from the list of viruses consisting of adenovirus, vaccinia virus, adeno associated virus, modified vaccinia ancara virus, and cytomegliavirus. It is understood that other viral vectors are in use for vaccine delivery. Specifically disclosed are each and every viral vector known in the art.

98. It is understood in the art that Candida express a CR3 like protein that has similar binding properties as CR2. The CR3 like protein appears to be involved in pathogenesis. Therefore, an embodiment of the invention is treating a subject with a fungal infection, wherein the treatment blocks fungal-"CR3" function as well as inhibits complement, comprising administering to a subject the compostition of the invention.

99. Disclosed are methods of the invention, wherein complement inhibitor can enhance the outcome of apoptosis-base therapy (e.g., gene therapy with adenovirus expressing Fas ligand).

100. Apoptosis occurring during normal development is non inflammatory and is involved in induction of immunological tolerance. Although apoptotic cell death can be inflammatory depending on how it is activated and in what cell types (for example, therapeutic agents that ligate Fas are able to induce inflammation), necrotic cell death results in a sustained and powerful inflammatory response mediated by released cell contents and by proinflammatory cytokines released by stimulated phagocytes. Apoptotic cells and vesicles are normally cleared by phagocytes, thus preventing the pro-inflammatory consequences of cell lysis. In this context, it has been shown that apoptotic cells and apoptotic bodies directly fix complement, and that complement can sustain an anti-inflammatory response due to opsonization and enhanced phagocytosis of apoptotic cells.

101. Inflammation is involved in non specific recruitment of immune cells that can influence innate and adaptive immune responses. Modulating complement activation during apoptosis-based tumor therapy to inhibit phagocytic uptake of apoptotic cells/bodies enhances the inflammatory/innate immune response within the tumor environment. In addition, apoptotic cells can be a source of immunogenic self antigens and uncleared apoptotic bodies can result in autoimmunization. In addition to creating an enhanced immuno-stimulatory environment, modulating complement at a site in which tumor cells have been induced to undergo apoptosis further augments or triggers specific immunity against a tumor to which the host is normally tolerant.

102. The disclosed compositions of the invention can act as CR2 and CR3 antagonists. Disclosed are methods of inhibiting complement activity via inhibition of CR2 comprising administering the composition of the invention to a subject. Also disclosed are are methods of inhibiting complement activity via inhibition of CR3 comprising administering the composition of the invention to a subject. As a CR2 antagonist can modulate immune response, a CR3 antagonist can have second anti-inflammatory mechanism of action since CR3 is integrin that binds endothelial ICAM1.

ICAM1 is expressed at sites of inflammation and is involved in leukocyte adhesion and diapedesis. In addition, ICAM1 expression is upregulated by complement activation products.

2. Methods of using the compositions to activate complement

103. Disclosed are methods of treating a condition affected by complement in a subject comprising administering to the subject the composition of the invention, wherein the composition will activate complement. It is understood that the administration of the composition to the subject can have the effect of, but is not limited to, reducing the symptoms of the condition, a reduction in the severity of the condition, or the complete ablation of the condition.

104. Disclosed are methods of enhancing complement-mediated damage comprising administering to a subject the composition of the invention, which activates complement.

Also disclosed are methods of the invention, wherein the condition is a cancer. The cancer can be selected from the group consisting of lymphomas (Hodgkins and non-Hodgkins), B cell lymphoma, T cell lymphoma, myeloid leukemia, leukemias, mycosis fungoides, carcinomas, carcinomas of solid tissues, squamous cell carcinomas, adenocarcinomas, sarcomas, gliomas, blastomas, neuroblastomas, plasmacytomas, histiocytomas, melanomas, adenomas, hypoxic tumours, myelomas, AIDS-related lymphomas or sarcomas, metastatic cancers, bladder cancer, brain cancer, nervous system cancer, squamous cell carcinoma of head and neck, neuroblastoma/glioblastoma, ovarian cancer, skin cancer, liver cancer, melanoma, squamous cell carcinomas of the mouth, throat, larynx, and lung, colon cancer, cervical cancer, cervical carcinoma, breast cancer, epithelial cancer, renal cancer, genitourinary cancer, pulmonary cancer, esophageal carcinoma, head and neck carcinoma, hematopoietic cancers, testicular cancer, colo-rectal cancers, prostatic cancer, or pancreatic cancer.

105. In one embodiment of the invention CR2 can target complement deposited on tumor cells as a result of administered anti-tumor antibodies, or as a result of a normally ineffective humoral immune response.

106. Thus the present complement activating composition can be administered in conjunction with anti-tumor antibodies. Examples of such anti-tumor antibodies are well known and include anti-PSMA monoclonal antibodies J591, PEQ226.5, and PM2P079.1 (Fracasso, G. et al., (2002) Prostate 53(1): 9-23); anti-Her2 antibody hu4D5 (Gerstner, R.B., et al., (2002) J. Mol. Biol. 321(5): 851-62); anti-disialosyl Gb5 monoclonal antibody 5F3 which can be used as an anti renal cell carcinoma antibody (Ito A. et al., (2001) Glycoconj. J. 18(6): 475-485); anti MAGE monoclonal antibody 57B (Antonescu, C.R. et al., (2002) Hum. Pathol. 33(2): 225-9); anti-cancer monoclonal antibody CLN-Ig (Kubo, O. et al., (2002) Nippon Rinsho. 60(3): 497-503); anti-Dalton's lymphoma associated antigen (DLAA) monoclonal antibody DLAB (Subbiah, K. et al., (2001) Indian J. Exp. Biol. 39(10): 993-7). The present composition can be administered before, concurrent with or after administration of the anti-tumor antibody, so long as the present composition is present at the tumor during the time when the antibody is also present at the tumor.

107. A representative but non-limiting list of cancers that the disclosed compositions can be used to treat is the following: lymphoma, B cell lymphoma, T cell lymphoma, mycosis fungoides, multiple myeloma, Hodgkin's Disease, myeloid leukemia, bladder cancer, brain cancer, nervous system cancer, head and neck cancer, squamous cell carcinoma of head and neck, kidney cancer, lung cancers such as small cell lung cancer and non-small cell lung cancer, urothelial carcinomas, adenocarcinomas, sarcomas, gliomas, high grade gliomas, blastomas, neuroblastomas, plasmacytomas, histiocytomas, , adenomas, hypoxic tumours, myelomas, AIDS-related lymphomas or sarcomas, metastatic cancers, neuroblastoma/glioblastoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, liver cancer, melanoma, squamous cell carcinomas of the mouth, throat, larynx, and lung, colon cancer, cervical cancer, cervical carcinoma, breast cancer, and epithelial cancer, renal cancer, genitourinary cancer, pulmonary cancer, esophageal carcinoma, head and neck carcinoma, large bowel cancer, hematopoietic cancers; testicular cancer; colon and rectal cancers, stomach cancer, prostatic cancer, Waldenstroms disease or pancreatic cancer.

108. The complement activating complisitions disclosed herein can also be used for the treatment of precancer conditions such as cervical and anal dysplasias, other dysplasias, severe dysplasias, hyperplasias, atypical hyperplasias, and neoplasias. Disclosed are methods of the invention, wherein the condition is a precancer conditions. It is understood that the composition will recognize antigens that are overexpressed on the surface of precancerous cells

109. Also disclosed are methods of using the complement activating compositions of the invention to treat viral infection. The viral infection can be selected from the list of viruses consisting of Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Papilomavirus, Respiratory syncytial virus, Adenovirus, Coxsackie virus, Dengue virus, Mumps virus, Poliovirus, Rabies virus, Rous sarcoma virus, Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency cirus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, and Human Immunodeficiency virus type-2.

110. Also disclosed are methods of using the complement activating compositions of the invention to treat a bacterial infection. Also disclosed are methods of the invention, wherein the bacterial infection can be selected from the list of bacterium consisting of *M. tuberculosis, M. bovis, M. bovis* strain BCG, BCG substrains, *M. avium, M. intracellulare, M. africanum, M. kansasii, M. marinum, M. ulcerans, M. avium* subspecies *paratuberculosis, Nocardia asteroides*, other *Nocardia* species, *Legionella pneumophila*, other *Legionella* species, *Salmonella typhi*, other *Salmonella* species, *Shigella* species, *Yersinia pestis, Pasteurella haemolytica,*

Pasteurella multocida, other *Pasteurella species, Actinobacillus pleuropneumoniae, Listeria monocytogenes, Listeria ivanovii, Brucella abortus,* other *Brucella* species, *Cowdria ruminantium, Chlamydia pneumoniae, Chlamydia trachomatis, Chlamydia psittaci, Coxiella burnetti,* other *Rickettsial* species, *Ehrlichia* species, *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus agalactiae, Bacillus anthracis, Escherichia coli, Vibrio cholerae, Campylobacter* species, *Neiserria meningitidis, Neiserria gonorrhea, Pseudomonas aeruginosa,* other *Pseudomonas* species, *Haemophilus influenzae, Haemophilus ducreyi,* other *Hemophilus species, Clostridium tetani,* other *Clostridium* species, *Yersinia enterolitica, and* other *Yersinia species.*

111. Also disclosed are methods of using the complement activating compositions of the invention to treat a parasitic infection. Also disclosed are methods of the invention, wherein the parasitic infection can be selected from the group consisting of *Toxoplasma gondii, Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae,* other *Plasmodium* species., *Trypanosoma brucei, Trypanosoma cruzi, Leishmania major,* other *Leishmania* species., *Schistosoma mansoni,* other *Schistosoma* species., and *Entamoeba histolytica.*

112. Also disclosed are methods of using the complement activiating compositions of the invention to treat a fungal infection. . Also disclosed are methods of the invention, wherein the fungal infection can be selected from the group consisting of *Candida albicans, Cryptococcus neoformans, Histoplama capsulatum, Aspergillus fumigatus, Coccidiodes immitis, Paracoccidiodes brasiliensis, Blastomyces dermitidis, Pneomocystis carnii, Penicillium marneffi, and Alternaria alternata.* In the methods of the invention, the subject can be a mammal. For example, the mammal can be a human, nonhuman primate, mouse, rat, pig, dog, cat, monkey, cow, or horse.

3. Methods of using the compositions as research tools

113. The disclosed compositions can be used in a variety of ways as research tools. For example, the disclosed compositions can be used to study inhibitor of complement activation.

114. The disclosed compositions can be used as diagnostic tools related to diseases associated with complement activation, such as cancer, viral infections, bacterial infections, parasitic infections, and fungal infections. CR2-fusion proteins will target a site of complement activation and a labeled CR2-fusion protein can diagnose conditions associated with complement activation. For example, a tumor-reactive antibody would activate complement on tumor cells, which CR2 could then target. The labeled CR2-Fc could then amplify the signal following antibody targeting.

D. Compositions

115. Disclosed are the components to be used to prepare the disclosed compositions as well as the compositions themselves to be used in the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference for each of the various individual and collective combinations and permutation of these compounds may not be explicitly made, each is specifically contemplated and described herein. For example, if a particular CR2, DAF, CD59, CR1, MCP, Crry, IgG1, IgM, IgG3, CVF is described, and/or a specific combination thereof is disclosed and discussed and/or a number of modifications that can be made to a number of molecules including the CR2, DAF, CD59, CR1, MCP, Crry, IgG1, IgM, IgG3, CVF, and/or combination thereof are discussed, specifically contemplated is each and every combination and permutation of CR2, DAF, CD59, CR1, MCP, Crry, IgG1, IgM, IgG3, CVF, or combination thereof and the modifications that are possible, unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional

steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods.

1. Sequence similarities

116. It is understood that as discussed herein the use of the terms homology and identity mean the same thing as similarity. Thus, for example, if the use of the word homology is used between two non-natural sequences it is understood that this is not necessarily indicating an evolutionary relationship between these two sequences, but rather is looking at the similarity or relatedness between their nucleic acid sequences. Many of the methods for determining homology between two evolutionarily related molecules are routinely applied to any two or more nucleic acids or proteins for the purpose of measuring sequence similarity regardless of whether they are evolutionarily related or not.

117. In general, it is understood that one way to define any known variants and derivatives or those that might arise, of the genes and proteins disclosed herein, is through defining the variants and derivatives in terms of homology to specific known sequences. This identity of particular sequences disclosed herein is also discussed elsewhere herein. For example SEQ ID NO: 25 sets forth a particular sequence of a CR2 and SEQ ID NO: 26 sets forth a particular sequence of the protein encoded by SEQ ID NO: 25, a CR2 protein. Specifically disclosed are variants of these and other genes and proteins herein disclosed which have at least, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 percent homology to the stated sequence. Those of skill in the art readily understand how to determine the homology of two proteins or nucleic acids, such as genes. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

118. Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. MoL Biol. 48: 443

(1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

119. The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment. It is understood that any of the methods typically can be used and that in certain instances the results of these various methods can differ, but the skilled artisan understands if identity is found with at least one of these methods, the sequences would be said to have the stated identity, and be disclosed herein.

120. For example, as used herein, a sequence recited as having a particular percent homology to another sequence refers to sequences that have the recited homology as calculated by any one or more of the calculation methods described above. For example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using the Zuker calculation method even if the first sequence does not have 80 percent homology to the second sequence as calculated by any of the other calculation methods. As another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using both the Zuker calculation method and the Pearson and Lipman calculation method even if the first sequence does not have 80 percent homology to the second sequence as calculated by the Smith and Waterman calculation method, the Needleman and Wunsch calculation method, the Jaeger calculation methods, or any of the other calculation methods. As yet another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using each of calculation methods (although, in practice, the different calculation methods will often result in different calculated homology percentages).

2. Nucleic acids

121. There are a variety of molecules disclosed herein that are nucleic acid based, including for example the nucleic acids that encode, for example CR2, DAF, CD59, CR1, MCP, Crry, IgG1, IgM, IgG3, CVF, CR2-DAF, DAF-CR2, CR2-CD59, CD59-CR2, CR2-CR1, CR1-CR2, CR2-MCP, MCP-CR2, CR2-Crry, Crry-CR2, CR2-anti-C5, CR2-IgG1 Fc (human), CR2-IgM Fc, CR2-IgG3 Fc (murine), or CR2-CVF, as well as various functional nucleic acids. The disclosed nucleic acids are made up of for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. It is understood that for example, when a vector is expressed in a cell, that the expressed mRNA will typically be made up of A, C, G, and U. Likewise, it is understood that if, for example, an antisense molecule is introduced into a cell or cell environment through for example exogenous delivery, it is advantagous that the antisense molecule be made up of nucleotide analogs that reduce the degradation of the antisense molecule in the cellular environment.

a) Nucleotides and related molecules

122. A nucleotide is a molecule that contains a base moiety, a sugar moiety and a phosphate moiety. Nucleotides can be linked together through their phosphate moieties and sugar moieties creating an internucleoside linkage. The base moiety of a nucleotide can be adenin-9-yl (A), cytosin-1-yl (C), guanin-9-yl (G), uracil-1-yl (U), and thymin-1-yl (T). The sugar moiety of a nucleotide is a ribose or a deoxyribose. The phosphate moiety of a nucleotide is pentavalent phosphate. An non-limiting example of a nucleotide would be 3'-AMP (3'-adenosine monophosphate) or 5'-GMP (5'-guanosine monophosphate).

123. A nucleotide analog is a nucleotide which contains some type of modification to either the base, sugar, or phosphate moieties. Modifications to the base moiety would include natural and synthetic modifications of A, C, G, and T/U as well as different purine or pyrimidine bases, such as uracil-5-yl (.psi.), hypoxanthin-9-yl (I), and 2-aminoadenin-9-yl. A modified base includes but is not limited to 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine,

2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Additional base modifications can be found for example in U.S. Pat. No. 3,687,808, Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613, and Sanghvi, Y. S., Chapter 15, Antisense Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B. ed., CRC Press, 1993. Certain nucleotide analogs, such as 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine can increase the stability of duplex formation. Often time base modifications can be combined with for example a sugar modifcation, such as 2'-O-methoxyethyl, to achieve unique properties such as increased duplex stability. There are numerous United States patents such as 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; and 5,681,941, which detail and describe a range of base modifications. Each of these patents is herein incorporated by reference.

124. Nucleotide analogs can also include modifications of the sugar moiety. Modifications to the sugar moiety would include natural modifications of the ribose and deoxy ribose as well as synthetic modifications. Sugar modifications include but are not limited to the following modifications at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C_1 to C_{10}, alkyl or C_2 to C_{10} alkenyl and alkynyl. 2' sugar modiifcations also include but are not limited to $-O[(CH_2)_n O]_m CH_3$, $-O(CH_2)_n OCH_3$, $-O(CH_2)_n NH_2$, $-O(CH_2)_n CH_3$, $-O(CH_2)_n -ONH_2$, and $-O(CH_2)_nON[(CH_2)_n CH_3)]_2$, where n and m are from 1 to about 10.

125. Other modifications at the 2' position include but are not limted to: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2 CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Similar modifications can also be made at other positions on the sugar, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Modified sugars would also include those that contain modifications at the bridging ring oxygen, such as CH_2 and S. Nucleotide sugar analogs can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. There are numerous United States patents that teach the preparation of such modified sugar structures such as 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference in its entirety.

126. Nucleotide analogs can also be modified at the phosphate moiety. Modified phosphate moieties include but are not limited to those that can be modified so that the linkage between two nucleotides contains a phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl and other alkyl phosphonates including 3'-alkylene phosphonate and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates. It is understood that these phosphate or modified phosphate linkage between two nucleotides can be through a 3'-5' linkage or a 2'-5' linkage, and the linkage can contain inverted polarity such as 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included. Numerous United States patents teach how to make and use nucleotides containing

modified phosphates and include but are not limited to, 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference.

127. It is understood that nucleotide analogs need only contain a single modification, but can also contain multiple modifications within one of the moieties or between different moieties.

128. Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

129. Nucleotide substitutes are nucleotides or nucleotide analogs that have had the phosphate moiety and/or sugar moieties replaced. Nucleotide substitutes do not contain a standard phosphorus atom. Substitutes for the phosphate can be for example, short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones;formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts. Numerous United States patents disclose how to make and use these types of phosphate replacements and include but are not limited to 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704;

5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

130. It is also understood in a nucleotide substitute that both the sugar and the phosphate moieties of the nucleotide can be replaced, by for example an amide type linkage (aminoethylglycine) (PNA). United States patents 5,539,082; 5,714,331;and 5,719,262 teach how to make and use PNA molecules, each of which is herein incorporated by reference. (See also Nielsen et al., Science, 1991, 254, 1497-1500).

131. It is also possible to link other types of molecules (conjugates) to nucleotides or nucleotide analogs to enhance for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556), cholic acid (Manoharan et al., Bioorg. Med. Chem. Let., 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660, 306-309; Manoharan et al., Bioorg. Med. Chem. Let., 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10, 1111-1118; Kabanov et al., FEBS Lett., 1990, 259, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654; Shea et al., Nucl. Acids Res., 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937. Numerous United States patents teach the preparation of such conjugates and include, but are not limited to U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735;

4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, each of which is herein incorporated by reference.

132. A Watson-Crick interaction is at least one interaction with the Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute. The Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute includes the C2, N1, and C6 positions of a purine based nucleotide, nucleotide analog, or nucleotide substitute and the C2, N3, C4 positions of a pyrimidine based nucleotide, nucleotide analog, or nucleotide substitute.

133. A Hoogsteen interaction is the interaction that takes place on the Hoogsteen face of a nucleotide or nucleotide analog, which is exposed in the major groove of duplex DNA. The Hoogsteen face includes the N7 position and reactive groups (NH2 or O) at the C6 position of purine nucleotides.

b) Sequences

134. There are a variety of sequences related to the CR2, DAF, CD59, CR1, MCP, Crry, IgG1, IgM, IgG3, CVF, CR2-DAF, DAF-CR2, CR2-CD59, CD59-CR2, CR2-CR1, CR1-CR2, CR2-MCP, MCP-CR2, CR2-Crry, Crry-CR2, CR2-IgG1 Fc (human), CR2-IgM Fc, CR2-IgG3 Fc (murine), or CR2-CVF genes having, for example, the sequences as disclosed herein or sequences available in the literature. These sequences and others are herein incorporated by reference in their entireties as well as for individual subsequences contained therein.

135. One particular sequence set forth in SEQ ID NO: 25 used herein, as an example, to exemplify the disclosed compositions and methods. It is understood that the description related to this sequence is applicable to any sequence related to CR2, CR2-DAF, DAF-CR2, CR2-CD59, CD59-CR2, CR2-CR1, CR1-CR2, CR2-MCP, MCP-CR2, CR2-Crry, Crry-CR2, CR2-IgG1 Fc (human), CR2-IgM Fc, CR2-IgG3 Fc (murine), or CR2-CVF unless specifically indicated otherwise. Those of skill in the art

understand how to resolve sequence discrepancies and differences and to adjust the compositions and methods relating to a particular sequence to other related sequences (i.e. sequences of CR2, DAF, CD59, CR1, MCP, Crry, IgG1, IgM, IgG3, CVF, CR2-DAF, DAF-CR2, CR2-CD59, CD59-CR2, CR2-CR1, CR1-CR2, CR2-MCP, MCP-CR2, CR2-Crry, Crry-CR2, CR2-IgG1 Fc (human), CR2-IgM Fc, CR2-IgG3 Fc (murine), or CR2-CVF). Primers and/or probes can be designed for any CR2, DAF, CD59, CR1, MCP, Crry, IgG1, IgM, IgG3, CVF, CR2-DAF, DAF-CR2, CR2-CD59, CD59-CR2, CR2-CR1, CR1-CR2, CR2-MCP, MCP-CR2, CR2-Crry, Crry-CR2, CR2-IgG1 Fc (human), CR2-IgM Fc, CR2-IgG3 Fc (murine), or CR2-CVF sequence given the information disclosed herein and known in the art.

3. Delivery of the compositions to cells

136. There are a number of compositions and methods which can be used to deliver the present fusion protein compositions, immunoconjugate compositions, and nucleic acid compositions to cells, either in vitro or in vivo. Compositions of the invention are preferably administered to a subject in a pharmaceutically acceptable carrier. Suitable carriers and their formulations are described in *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carriers include, but are not limited to, saline, water:oil emulsions, oil:water emulsions, water:oil:water emulsions, and Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of antibody being administered.

137. The compositions of the invention can be administered to the subject, patient, or cell by injection (e.g., intravenous, intraperitoneal, subcutaneous, intramuscular), or by other methods such as infusion that ensure its delivery to the bloodstream in an effective form. Local or intravenous injection is preferred.

138. Effective dosages and schedules for administering the compositions of the invention can be determined empirically, and making such determinations is within the skill in the art. Those skilled in the art will understand that the dosage of the compositions of the invention that must be administered will vary depending on, for example, the subject that will receive the composition, the route of administration, the particular type of composition used and other drugs being administered. A typical daily dosage of the compositions of the invention used alone might range from about 1 μg/kg to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above.

a) Nucleic acid based delivery systems

139. There are a number of compositions and methods which can be used to deliver nucleic acids to cells, either in vitro or in vivo. These methods and compositions can largely be broken down into two classes: viral based delivery systems and non-viral based delivery systems. For example, the nucleic acids can be delivered through a number of direct delivery systems such as, electroporation, lipofection, calcium phosphate precipitation, plasmids, viral vectors, viral nucleic acids, phage nucleic acids, phages, cosmids, or via transfer of genetic material in cells or carriers such as cationic liposomes. Appropriate means for transfection, including viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., Science, 247, 1465-1468, (1990); and Wolff, J. A. Nature, 352, 815-818, (1991)Such methods are well known in the art and readily adaptable for use with the compositions and methods described herein. In certain cases, the methods will be modifed to specifically function with large DNA molecules. Further, these methods can be used to target certain diseases and cell populations by using the targeting characteristics of the carrier.

140. Transfer vectors can be any nucleotide construction used to deliver genes into cells (e.g., a plasmid), or as part of a general strategy to deliver genes, e.g., as part of recombinant retrovirus or adenovirus (Ram et al. Cancer Res. 53:83-88, (1993)).

141. As used herein, plasmid or viral vectors are agents that transport the disclosed nucleic acids, such as SEQ ID NO: 25 into the cell without degradation and include a promoter yielding expression of the gene in the cells into which it is delivered. In some embodiments the CR2, DAF, CD59, CR1, MCP, Crry, IgG1, IgM, IgG3, CVF, CR2-DAF, DAF-CR2, CR2-CD59, CD59-CR2, CR2-CR1, CR1-CR2, CR2-MCP, MCP-CR2, CR2-Crry, Crry-CR2, CR2-IgG1 Fc (human), CR2-IgM Fc, CR2-IgG3 Fc (murine), or CR2-CVFs are derived from either a virus or a retrovirus. Viral vectors are, for example, Adenovirus, Adeno-associated virus, Herpes virus, Vaccinia virus, Polio virus, AIDS virus, neuronal trophic virus, Sindbis and other RNA viruses, including these viruses with the HIV backbone. Also preferred are any viral families which share the properties of these viruses which make them suitable for use as vectors. Retroviruses include Murine Maloney Leukemia virus, MMLV, and retroviruses that express the desirable properties of MMLV as a vector. Retroviral vectors are able to carry a larger genetic payload, i.e., a transgene or marker gene, than other viral vectors, and for this reason are a commonly used vector. However, they are not as useful in non-proliferating cells. Adenovirus vectors are relatively stable and easy to work with, have high titers, and can be delivered in aerosol formulation, and can transfect non-dividing cells. Pox viral vectors are large and have several sites for inserting genes, they are thermostable and can be stored at room temperature. A preferred embodiment is a viral vector which has been engineered so as to suppress the immune response of the host organism, elicited by the viral antigens. Preferred vectors of this type will carry coding regions for Interleukin 8 or 10.

142. Viral vectors can have higher transaction (ability to introduce genes) abilities than chemical or physical methods to introduce genes into cells. Typically, viral vectors contain, nonstructural early genes, structural late genes, an RNA polymerase III transcript, inverted terminal repeats necessary for replication and encapsidation, and promoters to control the transcription and replication of the viral

genome. When engineered as vectors, viruses typically have one or more of the early genes removed and a gene or gene/promotor cassette is inserted into the viral genome in place of the removed viral DNA. Constructs of this type can carry up to about 8 kb of foreign genetic material. The necessary functions of the removed early genes are typically supplied by cell lines which have been engineered to express the gene products of the early genes in trans.

(1) Retroviral Vectors

143. A retrovirus is an animal virus belonging to the virus family of Retroviridae, including any types, subfamilies, genus, or tropisms. Retroviral vectors, in general, are described by Verma, I.M., Retroviral vectors for gene transfer. In Microbiology-1985, American Society for Microbiology, pp. 229-232, Washington, (1985), which is incorporated by reference herein. Examples of methods for using retroviral vectors for gene therapy are described in U.S. Patent Nos. 4,868,116 and 4,980,286; PCT applications WO 90/02806 and WO 89/07136; and Mulligan, (Science 260:926-932 (1993)); the teachings of which are incorporated herein by reference.

144. A retrovirus is essentially a package which has packed into it nucleic acid cargo. The nucleic acid cargo carries with it a packaging signal, which ensures that the replicated daughter molecules will be efficiently packaged within the package coat. In addition to the package signal, there are a number of molecules which are needed in cis, for the replication, and packaging of the replicated virus. Typically a retroviral genome, contains the gag, pol, and env genes which are involved in the making of the protein coat. It is the gag, pol, and env genes which are typically replaced by the foreign DNA that it is to be transferred to the target cell. Retrovirus vectors typically contain a packaging signal for incorporation into the package coat, a sequence which signals the start of the gag transcription unit, elements necessary for reverse transcription, including a primer binding site to bind the tRNA primer of reverse transcription, terminal repeat sequences that guide the switch of RNA strands during DNA synthesis, a purine rich sequence 5' to the 3' LTR that serve as the priming site for the synthesis of the second strand of DNA synthesis, and specific sequences near the ends of the LTRs that enable the insertion of the DNA state of the retrovirus to insert

into the host genome. The removal of the gag, pol, and env genes allows for about 8 kb of foreign sequence to be inserted into the viral genome, become reverse transcribed, and upon replication be packaged into a new retroviral particle. This amount of nucleic acid is sufficient for the delivery of a one to many genes depending on the size of each transcript. It is preferable to include either positive or negative selectable markers along with other genes in the insert.

145. Since the replication machinery and packaging proteins in most retroviral vectors have been removed (gag, pol, and env), the vectors are typically generated by placing them into a packaging cell line. A packaging cell line is a cell line which has been transfected or transformed with a retrovirus that contains the replication and packaging machinery, but lacks any packaging signal. When the vector carrying the DNA of choice is transfected into these cell lines, the vector containing the gene of interest is replicated and packaged into new retroviral particles, by the machinery provided in cis by the helper cell. The genomes for the machinery are not packaged because they lack the necessary signals.

(2) Adenoviral Vectors

146. The construction of replication-defective adenoviruses has been described (Berkner et al., J. Virology 61:1213-1220 (1987); Massie et al., Mol. Cell. Biol. 6:2872-2883 (1986); Haj-Ahmad et al., J. Virology 57:267-274 (1986); Davidson et al., J. Virology 61:1226-1239 (1987); Zhang "Generation and identification of recombinant adenovirus by liposome-mediated transfection and PCR analysis" BioTechniques 15:868-872 (1993)). The benefit of the use of these viruses as vectors is that they are limited in the extent to which they can spread to other cell types, since they can replicate within an initial infected cell, but are unable to form new infectious viral particles. Recombinant adenoviruses have been shown to achieve high efficiency gene transfer after direct, in vivo delivery to airway epithelium, hepatocytes, vascular endothelium, CNS parenchyma and a number of other tissue sites (Morsy, J. Clin. Invest. 92:1580-1586 (1993); Kirshenbaum, J. Clin. Invest. 92:381-387 (1993); Roessler, J. Clin. Invest. 92:1085-1092 (1993); Moullier, Nature Genetics 4:154-159 (1993); La Salle, Science 259:988-990 (1993); Gomez-Foix, J. Biol. Chem.

267:25129-25134 (1992); Rich, Human Gene Therapy 4:461-476 (1993); Zabner, Nature Genetics 6:75-83 (1994); Guzman, Circulation Research 73:1201-1207 (1993); Bout, Human Gene Therapy 5:3-10 (1994); Zabner, Cell 75:207-216 (1993); Caillaud, Eur. J. Neuroscience 5:1287-1291 (1993); and Ragot, J. Gen. Virology 74:501-507 (1993)). Recombinant adenoviruses achieve gene transduction by binding to specific cell surface receptors, after which the virus is internalized by receptor-mediated endocytosis, in the same manner as wild type or replication-defective adenovirus (Chardonnet and Dales, Virology 40:462-477 (1970); Brown and Burlingham, J. Virology 12:386-396 (1973); Svensson and Persson, J. Virology 55:442-449 (1985); Seth, et al., J. Virol. 51:650-655 (1984); Seth, et al., Mol. Cell. Biol. 4:1528-1533 (1984); Varga et al., J. Virology 65:6061-6070 (1991); Wickham et al., Cell 73:309-319 (1993)).

147. A viral vector can be one based on an adenovirus which has had the E1 gene removed and these virons are generated in a cell line such as the human 293 cell line. In another preferred embodiment both the E1 and E3 genes are removed from the adenovirus genome.

(3) Adeno-asscociated viral vectors

148. Another type of viral vector is based on an adeno-associated virus (AAV). This defective parvovirus is a preferred vector because it can infect many cell types and is nonpathogenic to humans. AAV type vectors can transport about 4 to 5 kb and wild type AAV is known to stably insert into chromosome 19. Vectors which contain this site specific integration property are preferred. An especially preferred embodiment of this type of vector is the P4.1 C vector produced by Avigen, San Francisco, CA, which can contain the herpes simplex virus thymidine kinase gene, HSV-tk, and/or a marker gene, such as the gene encoding the green fluorescent protein, GFP.

149. In another type of AAV virus, the AAV contains a pair of inverted terminal repeats (ITRs) which flank at least one cassette containing a promoter which directs cell-specific expression operably linked to a heterologous gene. Heterologous in

this context refers to any nucleotide sequence or gene which is not native to the AAV or B19 parvovirus.

150. Typically the AAV and B19 coding regions have been deleted, resulting in a safe, noncytotoxic vector. The AAV ITRs, or modifications thereof, confer infectivity and site-specific integration, but not cytotoxicity, and the promoter directs cell-specific expression. United states Patent No. 6,261,834 is herein incorproated by reference for material related to the AAV vector.

151. The vectors of the present invention thus provide DNA molecules which are capable of integration into a mammalian chromosome without substantial toxicity.

152. The inserted genes in viral and retroviral usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and can contain upstream elements and response elements.

(4) Large payload viral vectors

153. Molecular genetic experiments with large human herpesviruses have provided a means whereby large heterologous DNA fragments can be cloned, propagated and established in cells permissive for infection with herpesviruses (Sun et al., Nature genetics 8: 33-41, 1994; Cotter and Robertson,.Curr Opin Mol Ther 5: 633-644, 1999). These large DNA viruses (herpes simplex virus (HSV) and Epstein-Barr virus (EBV), have the potential to deliver fragments of human heterologous DNA > 150 kb to specific cells. EBV recombinants can maintain large pieces of DNA in the infected B-cells as episomal DNA. Individual clones carried human genomic inserts up to 330 kb appeared genetically stable The maintenance of these episomes requires a specific EBV nuclear protein, EBNA1, constitutively expressed during infection with EBV. Additionally, these vectors can be used for transfection, where large amounts of protein can be generated transiently in vitro. Herpesvirus amplicon systems are also being used to package pieces of DNA > 220 kb and to infect cells that can stably maintain DNA as episomes.

154. Other useful systems include, for example, replicating and host-restricted non-replicating vaccinia virus vectors.

b) Non-nucleic acid based systems

155. The disclosed compositions can be delivered to the target cells in a variety of ways. For example, the compositions can be delivered through electroporation, or through lipofection, or through calcium phosphate precipitation. The delivery mechanism chosen will depend in part on the type of cell targeted and whether the delivery is occurring for example in vivo or in vitro.

156. Thus, the compositions can comprise, in addition to the disclosed CR2, DAF, CD59, CR1, MCP, Crry, IgG1, IgM, IgG3, CVF, CR2-DAF, DAF-CR2, CR2-CD59, CD59-CR2, CR2-CR1, CR1-CR2, CR2-MCP, MCP-CR2, CR2-Crry, Crry-CR2, CR2-IgG1 Fc (human), CR2-IgM Fc, CR2-IgG3 Fc (murine), or CR2-CVF or vectors for example, lipids such as liposomes, such as cationic liposomes (e.g., DOTMA, DOPE, DC-cholesterol) or anionic liposomes. Liposomes can further comprise proteins to facilitate targeting a particular cell, if desired. Administration of a composition comprising a compound and a cationic liposome can be administered to the blood afferent to a target organ or inhaled into the respiratory tract to target cells of the respiratory tract. Regarding liposomes, see, e.g., Brigham et al. *Am. J. Resp. Cell. Mol. Biol.* 1:95-100 (1989); Felgner et al. *Proc. Natl. Acad. Sci USA* 84:7413-7417 (1987); U.S. Pat. No.4,897,355. Furthermore, the compound can be administered as a component of a microcapsule that can be targeted to specific cell types, such as macrophages, or where the diffusion of the compound or delivery of the compound from the microcapsule is designed for a specific rate or dosage.

157. In the methods described above which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), delivery of the compositions to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO-BRL, Inc., Gaithersburg, MD), SUPERFECT (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, WI), as well as other liposomes

developed according to procedures standard in the art. In addition, the nucleic acid or vector of this invention can be delivered *in vivo* by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, CA) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp., Tucson, AZ).

158. The materials can be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These can be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). These techniques can be used for a variety of other speciifc cell types. Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of

receptor-mediated endocytosis has been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

159. Nucleic acids that are delivered to cells which are to be integrated into the host cell genome, typically contain integration sequences. These sequences are often viral related sequences, particularly when viral based systems are used. These viral intergration systems can also be incorporated into nucleic acids which are to be delivered using a non-nucleic acid based system of deliver, such as a liposome, so that the nucleic acid contained in the delivery system can be come integrated into the host genome.

160. Other general techniques for integration into the host genome include, for example, systems designed to promote homologous recombination with the host genome. These systems typically rely on sequence flanking the nucleic acid to be expressed that has enough homology with a target sequence within the host cell genome that recombination between the vector nucleic acid and the target nucleic acid takes place, causing the delivered nucleic acid to be integrated into the host genome. These systems and the methods necessary to promote homologous recombination are known to those of skill in the art.

c) In vivo/ex vivo

161. As described above, the compositions can be administered in a pharmaceutically acceptable carrier and can be delivered to the subject=s cells *in vivo* and/or *ex vivo* by a variety of mechanisms well known in the art (e.g., uptake of naked DNA, liposome fusion, intramuscular injection of DNA via a gene gun, endocytosis and the like).

162. If *ex vivo* methods are employed, cells or tissues can be removed and maintained outside the body according to standard protocols well known in the art. The compositions can be introduced into the cells via any gene transfer mechanism, such as, for example, calcium phosphate mediated gene delivery, electroporation, microinjection or proteoliposomes. The transduced cells can then be infused (e.g., in a pharmaceutically acceptable carrier) or homotopically transplanted back into the subject

per standard methods for the cell or tissue type. Standard methods are known for transplantation or infusion of various cells into a subject.

4. Expression systems

163. The nucleic acids that are delivered to cells typically contain expression controlling systems. For example, the inserted genes in viral and retroviral systems usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and can contain upstream elements and response elements.

a) Viral Promoters and Enhancers

164. Preferred promoters controlling transcription from vectors in mammalian host cells can be obtained from various sources, for example, the genomes of viruses such as: polyoma, Simian Virus 40 (SV40), adenovirus, retroviruses, hepatitis-B virus and most preferably cytomegalovirus, or from heterologous mammalian promoters, e.g. beta actin promoter. The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication (Fiers et al., Nature, 273: 113 (1978)). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment (Greenway, P.J. et al., Gene 18: 355-360 (1982)). Of course, promoters from the host cell or related species also are useful herein.

165. Enhancer generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' (Laimins, L. et al., Proc. Natl. Acad. Sci. 78: 993 (1981)) or 3' (Lusky, M.L., et al., Mol. Cell Bio. 3: 1108 (1983)) to the transcription unit. Furthermore, enhancers can be within an intron (Banerji, J.L. et al., Cell 33: 729 (1983)) as well as within the coding sequence itself (Osborne, T.F., et al., Mol. Cell Bio. 4: 1293 (1984)). They are usually between 10 and 300 bp in length, and they function in cis. Enhancers f unction to increase transcription from nearby promoters. Enhancers also often contain response elements that mediate the regulation of transcription. Promoters can also contain response elements that

mediate the regulation of transcription. Enhancers often determine the regulation of expression of a gene. While many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, -fetoprotein and insulin), typically one will use an enhancer from a eukaryotic cell virus for general expression. Preferred examples are the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

166. The promotor and/or enhancer can be specifically activated either by light or specific chemical events which trigger their function. Systems can be regulated by reagents such as tetracycline and dexamethasone. There are also ways to enhance viral vector gene expression by exposure to irradiation, such as gamma irradiation, or alkylating chemotherapy drugs.

167. In certain embodiments the promoter and/or enhancer region can act as a constitutive promoter and/or enhancer to maximize expression of the region of the transcription unit to be transcribed. In certain constructs the promoter and/or enhancer region be active in all eukaryotic cell types, even if it is only expressed in a particular type of cell at a particular time. A preferred promoter of this type is the CMV promoter (650 bases). Other preferred promoters are SV40 promoters, cytomegalovirus (full length promoter), and retroviral vector LTF.

168. It has been shown that all specific regulatory elements can be cloned and used to construct expression vectors that are selectively expressed in specific cell types such as melanoma cells. The glial fibrillary acetic protein (GFAP) promoter has been used to selectively express genes in cells of glial origin.

169. Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells) can also contain sequences necessary for the termination of transcription which can affect mRNA expression. These regions are transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding tissue factor protein. The 3' untranslated regions also include transcription termination sites. It is preferred that the transcription unit also contains a polyadenylation region. One benefit of this region is that it increases the likelihood that

the transcribed unit will be processed and transported like mRNA. The identification and use of polyadenylation signals in expression constructs is well established. It is preferred that homologous polyadenylation signals be used in the transgene constructs. In certain transcription units, the polyadenylation region is derived from the SV40 early polyadenylation signal and consists of about 400 bases. It is also preferred that the transcribed units contain other standard sequences alone or in combination with the above sequences improve expression from, or stability of, the construct.

b) Markers

170. The viral vectors can include nucleic acid sequence encoding a marker product. This marker product is used to determine if the gene has been delivered to the cell and once delivered is being expressed. Preferred marker genes are the *E. Coli* lacZ gene, which encodes ß-galactosidase, and green fluorescent protein.

171. In some embodiments the marker can be a selectable marker. Examples of suitable selectable markers for mammalian cells are dihydrofolate reductase (DHFR), thymidine kinase, neomycin, neomycin analog G418, hydromycin, and puromycin. When such selectable markers are successfully transferred into a mammalian host cell, the transformed mammalian host cell can survive if placed under selective pressure. There are two widely used distinct categories of selective regimes. The first category is based on a cell's metabolism and the use of a mutant cell line which lacks the ability to grow independent of a supplemented media. Two examples are: CHO DHFR- cells and mouse LTK- cells. These cells lack the ability to grow without the addition of such nutrients as thymidine or hypoxanthine. Because these cells lack certain genes necessary for a complete nucleotide synthesis pathway, they cannot survive unless the missing nucleotides are provided in a supplemented media. An alternative to supplementing the media is to introduce an intact DHFR or TK gene into cells lacking the respective genes, thus altering their growth requirements. Individual cells which were not transformed with the DHFR or TK gene will not be capable of survival in non-supplemented media.

172. The second category is dominant selection which refers to a selection scheme used in any cell type and does not require the use of a mutant cell line. These

schemes typically use a drug to arrest growth of a host cell. Those cells which have a novel gene would express a protein conveying drug resistance and would survive the selection. Examples of such dominant selection use the drugs neomycin, (Southern P. and Berg, P., J. Molec. Appl. Genet. 1: 327 (1982)), mycophenolic acid, (Mulligan, R.C. and Berg, P. Science 209: 1422 (1980)) or hygromycin, (Sugden, B. et al., Mol. Cell. Biol. 5: 410-413 (1985)). The three examples employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid) or hygromycin, respectively. Others include the neomycin analog G418 and puramycin.

5. Peptides

a) Protein variants

173. As discussed herein there are numerous variants of the CR2, DAF, CD59, CR1, MCP, Crry, IgG1, IgM, IgG3, CVF, CR2-DAF, DAF-CR2, CR2-CD59, CD59-CR2, CR2-CR1, CR1-CR2, CR2-MCP, MCP-CR2, CR2-Crry, Crry-CR2, CR2-IgG1 Fc (human), CR2-IgM Fc, CR2-IgG3 Fc (murine), and CR2-CVF protein that are known and herein contemplated. In addition, to the known functional CR2, DAF, CD59, CR1, MCP, Crry, IgG1, IgM, IgG3, CVF, CR2-DAF, DAF-CR2, CR2-CD59, CD59-CR2, CR2-CR1, CR1-CR2, CR2-MCP, MCP-CR2, CR2-Crry, Crry-CR2, CR2-IgG1 Fc (human), CR2-IgM Fc, CR2-IgG3 Fc (murine), and CR2-CVF strain variants, there are derivatives of the CR2, DAF, CD59, CR1, MCP, Crry, IgG1, IgM, IgG3, CVF, CR2-DAF, DAF-CR2, CR2-CD59, CD59-CR2, CR2-CR1, CR1-CR2, CR2-MCP, MCP-CR2, CR2-Crry, Crry-CR2, CR2-IgG1 Fc (human), CR2-IgM Fc, CR2-IgG3 Fc (murine), and CR2-CVF proteins which also function in the disclosed methods and compositions. Protein variants and derivatives are well understood to those of skill in the art and can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Immunogenic fusion protein

derivatives, such as those described in the examples, are made by fusing a polypeptide sufficiently large to confer immunogenicity to the target sequence by cross-linking in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e. a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof can be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Tables 1 and 2 and are referred to as conservative substitutions.

174. TABLE 1:Amino Acid Abbreviations

Amino Acid	Abbreviations
alanine	Ala A
allosoleucine	AIle
arginine	Arg R
asparagine	Asn N
aspartic acid	Asp D
cysteine	Cys C
glutamic acid	Glu E
glutamine	Gln Q
glycine	Gly G
histidine	His H

Amino Acid	Abbreviations
isolelucine	Ile I
leucine	Leu L
lysine	Lys K
phenylalanine	Phe F
proline	Pro P
pyroglutamic acidp	pGlu
serine	Ser S
threonine	Thr T
tyrosine	Tyr Y
tryptophan	Trp W
valine	Val V

TABLE 2:Amino Acid Substitutions
Original Residue Exemplary Conservative Substitutions, others are known in the art.
Ala; Ser
Arg;Lys; Gln
Asn; Gln; His
Asp; Glu
Cys; Ser
Gln; Asn, Lys
Glu; Asp
Gly; Pro
His; Asn; Gln
Ile; Leu; Val
Leu; Ile; Val
Lys; Arg; Gln;
Met; Leu; Ile
Phe; Met; Leu; Tyr
Ser; Thr
Thr; Ser
Trp; Tyr
Tyr; Trp; Phe
Val; Ile; Leu

175. Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those in Table 2, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. The substitutions which in general are expected to

produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

176. For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the mosaic polypeptides provided herein.

177. Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also may be desirable. Deletions or substitutions of potential proteolysis sites, e.g. Arg, is accomplished for example by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

178. Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and asparyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco pp

79-86 [1983]), acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

179. It is understood that one way to define the variants and derivatives of the disclosed proteins herein is through defining the variants and derivatives in terms of homology/identity to specific known sequences. For example, SEQ ID NO: 26 sets forth a particular sequence of CR2 and SEQ ID NO: 2 sets forth a particular sequence of a DAF protein. Specifically disclosed are variants of these and other proteins herein disclosed which have at least, 70% or 75% or 80% or 85% or 90% or 95% homology to the stated sequence. Those of skill in the art readily understand how to determine the homology of two proteins. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

180. Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. MoL Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

181. The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment.

182. It is understood that the description of conservative mutations and homology can be combined together in any combination, such as embodiments that have at least 70% homology to a particular sequence wherein the variants are conservative mutations.

183. As this specification discusses various proteins and protein sequences it is understood that the nucleic acids that can encode those protein sequences are also

disclosed. This would include all degenerate sequences related to a specific protein sequence, i.e. all nucleic acids having a sequence that encodes one particular protein sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the protein sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed protein sequence. For example, one of the many nucleic acid sequences that can encode the protein sequence set forth in SEQ ID NO:26 is set forth in SEQ ID NO:25. In addition, for example, a disclosed conservative derivative of SEQ ID NO:26 is shown in SEQ ID NO: 29, where the isoleucine (I) at position 9 is changed to a valine (V). It is understood that for this mutation all of the nucleic acid sequences that encode this particular derivative of any of the disclosed sequences are also disclosed. It is also understood that while no amino acid sequence indicates what particular DNA sequence encodes that protein within an organism, where particular variants of a disclosed protein are disclosed herein, the known nucleic acid sequence that encodes that protein from which that protein arises is also known and herein disclosed and described.

6. Antibodies

a) Antibodies Generally

184. The term "antibodies" is used herein in a broad sense and includes both polyclonal and monoclonal antibodies. In addition to intact immunoglobulin molecules, also included in the term "antibodies" are fragments or polymers of those immunoglobulin molecules, and human or humanized versions of immunoglobulin molecules or fragments thereof, as described herein. The antibodies are tested for their desired activity using the *in vitro* assays described herein, or by analogous methods, after which their *in vivo* therapeutic and/or prophylactic activities are tested according to known clinical testing methods.

185. As used herein, the term "antibody" encompasses, but is not limited to, whole immunoglobulin (i.e., an intact antibody) of any class. Native antibodies are usually heterotetrameric glycoproteins, composed of two identical light (L) chains and two identical heavy (H) chains. Typically, each light chain is linked to a heavy chain by

one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V(H)) followed by a number of constant domains. Each light chain has a variable domain at one end (V(L)) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains. The light chains of antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (k) and lambda (l), based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of human immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these can be further divided into subclasses (isotypes), e.g., IgG-1, IgG-2, IgG-3, and IgG-4; IgA-1 and IgA-2. One skilled in the art would recognize the comparable classes for mouse. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively.

186. The term “variable” is used herein to describe certain portions of the variable domains that differ in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not usually evenly distributed through the variable domains of antibodies. It is typically concentrated in three segments called complementarity determining regions (CDRs) or hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of the variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a b-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the b-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the

antigen binding site of antibodies (see Kabat E. A. et al., "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1987)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

187. As used herein, the term "antibody or fragments thereof" encompasses chimeric antibodies and hybrid antibodies, with dual or multiple antigen or epitope specificities, and fragments, such as scFv, sFv, F(ab')2, Fab', Fab and the like, including hybrid fragments. Thus, fragments of the antibodies that retain the ability to bind their specific antigens are provided. For example, fragments of antibodies which maintain complement binding activity binding activity are included within the meaning of the term "antibody or fragment thereof." Such antibodies and fragments can be made by techniques known in the art and can be screened for specificity and activity according to the methods set forth in the Examples and in general methods for producing antibodies and screening antibodies for specificity and activity (See Harlow and Lane. Antibodies, A Laboratory Manual. Cold Spring Harbor Publications, New York, (1988)).

188. Also included within the meaning of "antibody or fragments thereof" are conjugates of antibody fragments and antigen binding proteins (single chain antibodies) as described, for example, in U.S. Pat. No. 4,704,692, the contents of which are hereby incorporated by reference.

189. As used herein, the term "antibody" or "antibodies" can also refer to a human antibody and/or a humanized antibody. Many non-human antibodies (e.g., those derived from mice, rats, or rabbits) are naturally antigenic in humans, and thus can give rise to undesirable immune responses when administered to humans. Therefore, the use of human or humanized antibodies in the methods of the invention serves to lessen the chance that an antibody administered to a human will evoke an undesirable immune response.

190. Optionally, the antibodies are generated in other species and "humanized" for administration in humans. Humanized forms of non-human (e.g.,

murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fc, scFv, sFv, Fv, Fab, Fab', F(ab')2, or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can also comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)).

191. Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and

possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

192. The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important in order to reduce antigenicity. According to the "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims et al., J. Immunol., 151:2296 (1993) and Chothia et al., J. Mol. Biol., 196:901 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework can be used for several different humanized antibodies (Carter et al., Proc. Natl. Acad. Sci. USA, 89:4285 (1992); Presta et al., J. Immunol., 151:2623 (1993)).

193. It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequence so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding (see, WO 94/04679, published 3 March 1994).

194. The Fab fragments produced in the antibody digestion also contain the constant domains of the light chain and the first constant domain of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain domain including one or more cysteines from the antibody hinge region. The F(ab')2 fragment is a bivalent fragment comprising two Fab' fragments linked by a disulfide bridge at the hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. Antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

195. An isolated immunogenically specific paratope or fragment of the antibody is also provided. A specific immunogenic epitope of the antibody can be isolated from the whole antibody by chemical or mechanical disruption of the molecule. The purified fragments thus obtained are tested to determine their immunogenicity and specificity by the methods taught herein. Immunoreactive paratopes of the antibody, optionally, are synthesized directly. An immunoreactive fragment is defined as an amino acid sequence of at least about two to five consecutive amino acids derived from the antibody amino acid sequence.

196. One method of producing proteins comprising the antibodies of the present invention is to link two or more peptides or polypeptides together by protein chemistry techniques. For example, peptides or polypeptides can be chemically synthesized using currently available laboratory equipment using either Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (tert -butyloxycarbonoyl) chemistry. (Applied Biosystems, Inc., Foster City, CA). One skilled in the art can readily appreciate that a peptide or polypeptide corresponding to the antibody of the present invention, for example, can be synthesized by standard chemical reactions. For example, a peptide or polypeptide can be synthesized and not cleaved from its synthesis resin whereas the other fragment of an antibody can be synthesized and subsequently cleaved from the resin, thereby exposing a terminal group which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently

joined via a peptide bond at their carboxyl and amino termini, respectively, to form an antibody, or fragment thereof. (Grant GA (1992) Synthetic Peptides: A User Guide. W.H. Freeman and Co., N.Y. (1992); Bodansky M and Trost B., Ed. (1993) Principles of Peptide Synthesis. Springer-Verlag Inc., NY. Alternatively, the peptide or polypeptide is independently synthesized in vivo as described above. Once isolated, these independent peptides or polypeptides can be linked to form an antibody or fragment thereof via similar peptide condensation reactions.

197. For example, enzymatic ligation of cloned or synthetic peptide segments allow relatively short peptide fragments to be joined to produce larger peptide fragments, polypeptides or whole protein domains (Abrahmsen L et al., Biochemistry, 30:4151 (1991)). Alternatively, native chemical ligation of synthetic peptides can be utilized to synthetically construct large peptides or polypeptides from shorter peptide fragments. This method consists of a two step chemical reaction (Dawson et al. Synthesis of Proteins by Native Chemical Ligation. Science, 266:776-779 (1994)). The first step is the chemoselective reaction of an unprotected synthetic peptide-alpha-thioester with another unprotected peptide segment containing an amino-terminal Cys residue to give a thioester-linked intermediate as the initial covalent product. Without a change in the reaction conditions, this intermediate undergoes spontaneous, rapid intramolecular reaction to form a native peptide bond at the ligation site. Application of this native chemical ligation method to the total synthesis of a protein molecule is illustrated by the preparation of human interleukin 8 (IL-8) (Baggiolini M et al. (1992) FEBS Lett. 307:97-101; Clark-Lewis I et al., J.Biol.Chem., 269:16075 (1994); Clark-Lewis I et al., Biochemistry, 30:3128 (1991); Rajarathnam K et al., Biochemistry 33:6623-30 (1994)).

198. Alternatively, unprotected peptide segments are chemically linked where the bond formed between the peptide segments as a result of the chemical ligation is an unnatural (non-peptide) bond (Schnolzer, M et al. Science, 256:221 (1992)). This technique has been used to synthesize analogs of protein domains as well as large amounts of relatively pure proteins with full biological activity (deLisle Milton RC et

al., Techniques in Protein Chemistry IV. Academic Press, New York, pp. 257-267 (1992)).

199. The invention also provides fragments of antibodies which have bioactivity. The polypeptide fragments of the present invention can be recombinant proteins obtained by cloning nucleic acids encoding the polypeptide in an expression system capable of producing the polypeptide fragments thereof, such as an adenovirus or baculovirus expression system. For example, one can determine the active domain of an antibody from a specific hybridoma that can cause a biological effect associated with the interaction of the antibody with an Fc receptor. For example, amino acids found to not contribute to either the activity or the binding specificity or affinity of the antibody can be deleted without a loss in the respective activity. For example, in various embodiments, amino or carboxy-terminal amino acids are sequentially removed from either the native or the modified non-immunoglobulin molecule or the immunoglobulin molecule and the respective activity assayed in one of many available assays. In another example, a fragment of an antibody comprises a modified antibody wherein at least one amino acid has been substituted for the naturally occurring amino acid at a specific position, and a portion of either amino terminal or carboxy terminal amino acids, or even an internal region of the antibody, has been replaced with a polypeptide fragment or other moiety, such as biotin, which can facilitate in the purification of the modified antibody.

200. The fragments, whether attached to other sequences or not, include insertions, deletions, substitutions, or other selected modifications of particular regions or specific amino acids residues, provided the activity of the fragment is not significantly altered or impaired compared to the nonmodified antibody or antibody fragment. These modifications can provide for some additional property, such as to remove or add amino acids capable of disulfide bonding, to increase its bio-longevity, to alter its secretory characteristics, etc. In any case, the fragment must possess a bioactive property, such as binding activity, regulation of binding at the binding domain, etc. Functional or active regions of the antibody can be identified by mutagenesis of a specific region of the protein, followed by expression and testing of

the expressed polypeptide. Such methods are readily apparent to a skilled practitioner in the art and can include site-specific mutagenesis of the nucleic acid encoding the antigen. (Zoller MJ et al. Nucl. Acids Res. 10:6487-500 (1982).

201. A variety of immunoassay formats can be used to select antibodies that selectively bind with a particular protein, variant, or fragment. For example, solid-phase ELISA immunoassays are routinely used to select antibodies selectively immunoreactive with a protein, protein variant, or fragment thereof. See Harlow and Lane. Antibodies, A Laboratory Manual. Cold Spring Harbor Publications, New York, (1988), for a description of immunoassay formats and conditions that could be used to determine selective binding. The binding affinity of a monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson et al., Anal. Biochem., 107:220 (1980).

202. Also provided is an antibody reagent kit comprising containers of the monoclonal antibody or fragment thereof of the invention and one or more reagents for detecting binding of the antibody or fragment thereof to the Fc receptor molecule. The reagents can include, for example, fluorescent tags, enzymatic tags, or other tags. The reagents can also include secondary or tertiary antibodies or reagents for enzymatic reactions, wherein the enzymatic reactions produce a product that can be visualized.

b) Human antibodies

203. The human antibodies of the invention can be prepared using any technique. Examples of techniques for human monoclonal antibody production include those described by Cole et al. (*Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77, 1985) and by Boerner et al. (*J. Immunol.*, 147(1):86-95, 1991). Human antibodies of the invention (and fragments thereof) can also be produced using phage display libraries (Hoogenboom et al., *J. Mol. Biol.*, 227:381, 1991; Marks et al., *J. Mol. Biol.*, 222:581, 1991).

204. The human antibodies of the invention can also be obtained from transgenic animals. For example, transgenic, mutant mice that are capable of producing a full repertoire of human antibodies, in response to immunization, have been described (see, e.g., Jakobovits et al., *Proc. Natl. Acad. Sci. USA*, 90:2551-255 (1993); Jakobovits

et al., *Nature*, 362:255-258 (1993); Bruggermann et al., *Year in Immunol.*, 7:33 (1993)). Specifically, the homozygous deletion of the antibody heavy chain joining region (J*(H)*) gene in these chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production, and the successful transfer of the human germ-line antibody gene array into such germ-line mutant mice results in the production of human antibodies upon antigen challenge. Antibodies having the desired activity are selected using Env-CD4-co-receptor complexes as described herein.

c) Administration of antibodies

205. Antibodies of the invention are preferably administered to a subject in a pharmaceutically acceptable carrier. Suitable carriers and their formulations are described in *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include, but are not limited to, saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of antibody being administered.

206. The antibodies can be administered to the subject, patient, or cell by injection (e.g., intravenous, intraperitoneal, subcutaneous, intramuscular), or by other methods such as infusion that ensure its delivery to the bloodstream in an effective form. Local or intravenous injection is preferred.

207. Effective dosages and schedules for administering the antibodies can be determined empirically, and making such determinations is within the skill in the art. Those skilled in the art will understand that the dosage of antibodies that must be administered will vary depending on, for example, the subject that will receive the

antibody, the route of administration, the particular type of antibody used and other drugs being administered. Guidance in selecting appropriate doses for antibodies is found in the literature on therapeutic uses of antibodies, e.g., Handbook of Monoclonal Antibodies, Ferrone et al., eds., Noges Publications, Park Ridge, N.J., (1985) ch. 22 and pp. 303-357; Smith et al., Antibodies in Human Diagnosis and Therapy, Haber et al., eds., Raven Press, New York (1977) pp. 365-389. A typical daily dosage of the antibody used alone might range from about 1 μg/kg to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above.

208. Following administration of an antibody for treating, inhibiting, or preventing an HIV infection, the efficacy of the therapeutic antibody can be assessed in various ways well known to the skilled practitioner. For instance, one of ordinary skill in the art will understand that an antibody of the invention is efficacious in treating or inhibiting an HIV infection in a subject by observing that the antibody reduces viral load or prevents a further increase in viral load. Viral loads can be measured by methods that are known in the art, for example, using polymerase chain reaction assays to detect the presence of HIV nucleic acid or antibody assays to detect the presence of HIV protein in a sample (e.g., but not limited to, blood) from a subject or patient, or by measuring the level of circulating anti-HIV antibody levels in the patient. Efficacy of the antibody treatment can also be determined by measuring the number of $CD4^+$ T cells in the HIV-infected subject. An antibody treatment that inhibits an initial or further decrease in $CD4^+$ T cells in an HIV-positive subject or patient, or that results in an increase in the number of $CD4^+$ T cells in the HIV-positive subject, is an efficacious antibody treatment.

d) Nucleic acid approaches for antibody delivery

209. The compositions of the invention can also be administered to patients or subjects as a nucleic acid preparation (e.g., DNA or RNA) that encodes the antibody or antibody fragment, such that the patient's or subject's own cells take up the nucleic acid and produce and secrete the encoded composition (e.g., CR2-DAF, DAF-CR2, CR2-CD59, CD59-CR2, CR2-CR1, CR1-CR2, CR2-MCP, MCP-CR2, CR2-Crry, Crry-CR2, CR2-IgG1 Fc (human), CR2-IgM Fc, CR2-IgG3 Fc (murine), or CR2-CVF).

e) **Nucleic Acid Delivery**

210. In the methods described above which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), the nucleic acids of the present invention can be in the form of naked DNA or RNA, or the nucleic acids can be in a vector for delivering the nucleic acids to the cells, whereby the antibody-encoding DNA fragment is under the transcriptional regulation of a promoter, as would be well understood by one of ordinary skill in the art. The vector can be a commercially available preparation, such as an adenovirus vector (Quantum Biotechnologies, Inc. (Laval, Quebec, Canada). Delivery of the nucleic acid or vector to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO-BRL, Inc., Gaithersburg, MD), SUPERFECT (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, WI), as well as other liposomes developed according to procedures standard in the art. In addition, the nucleic acid or vector of this invention can be delivered *in vivo* by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, CA) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp., Tucson, AZ).

211. As one example, vector delivery can be via a viral system, such as a retroviral vector system which can package a recombinant retroviral genome (see e.g., Pastan et al., *Proc. Natl. Acad. Sci. U.S.A.* 85:4486, 1988; Miller et al., *Mol. Cell. Biol.* 6:2895, 1986). The recombinant retrovirus can then be used to infect and thereby deliver to the infected cells nucleic acid encoding a broadly neutralizing antibody (or active fragment thereof) of the invention. The exact method of introducing the altered nucleic acid into mammalian cells is, of course, not limited to the use of retroviral vectors. Other techniques are widely available for this procedure including the use of adenoviral vectors (Mitani et al., *Hum. Gene Ther.* 5:941-948, 1994), adeno-associated viral (AAV) vectors (Goodman et al., *Blood* 84:1492-1500, 1994), lentiviral vectors (Naidini et al., *Science* 272:263-267, 1996), pseudotyped retroviral vectors (Agrawal et al., *Exper. Hematol.* 24:738-747, 1996). Physical transduction techniques can also be

used, such as liposome delivery and receptor-mediated and other endocytosis mechanisms (see, for example, Schwartzenberger et al., *Blood* 87:472-478, 1996). This invention can be used in conjunction with any of these or other commonly used gene transfer methods.

212. As one example, if the complement modulating construct-encoding nucleic acid of the invention is delivered to the cells of a subject in an adenovirus vector, the dosage for administration of adenovirus to humans can range from about 10^7 to 10^9 plaque forming units (pfu) per injection but can be as high as 10^{12} pfu per injection (Crystal, *Hum. Gene Ther.* 8:985-1001, 1997; Alvarez and Curiel, *Hum. Gene Ther.* 8:597-613, 1997). A subject can receive a single injection, or, if additional injections are necessary, they can be repeated at six month intervals (or other appropriate time intervals, as determined by the skilled practitioner) for an indefinite period and/or until the efficacy of the treatment has been established.

213. Parenteral administration of the nucleic acid or vector of the present invention, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein. For additional discussion of suitable formulations and various routes of administration of therapeutic compounds, see, e.g., *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995.

7. Pharmaceutical carriers/Delivery of pharamceutical products

214. As described above, the compositions can also be administered *in vivo* in a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material can be administered to a subject, along with the nucleic acid or vector, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The

carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

215. The compositions can be administered orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, topically or the like, although topical intranasal administration or administration by inhalant is typically preferred. As used herein, "topical intranasal administration" means delivery of the compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the nucleic acid or vector. The latter is effective when a large number of animals is to be treated simultaneously. Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation. The exact amount of the compositions required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the allergic disorder being treated, the particular nucleic acid or vector used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

216. Parenteral administration of the composition, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein.

217. The materials can be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These can be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of

the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis has been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

a) Pharmaceutically Acceptable Carriers

218. The compositions, including antibodies, can be used therapeutically in combination with a pharmaceutically acceptable carrier.

219. Pharmaceutical carriers are known to those skilled in the art. These most typically would be standard carriers for administration of drugs to humans, including solutions such as sterile water, saline, and buffered solutions at physiological

pH. The compositions can be administered intramuscularly or subcutaneously. Other compounds will be administered according to standard procedures used by those skilled in the art.

220. Pharmaceutical compositions can include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the molecule of choice. Pharmaceutical compositions can also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like.

221. The pharmaceutical composition can be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration can be topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection. The disclosed antibodies can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally.

222. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives can also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

223. Formulations for topical administration can include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

224. Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable.

225. Some of the compositions can be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

b) Therapeutic Uses

226. The dosage ranges for the administration of the compositions are those large enough to produce the desired effect in which the symptoms disorder are effected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counterindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days.

8. Computer readable mediums

227. It is understood that the disclosed nucleic acids and proteins can be represented as a sequence consisting of the nucleotides of amino acids. There are a variety of ways to display these sequences, for example the nucleotide guanosine can be represented by G or g. Likewise the amino acid valine can be represented by Val or V. Those of skill in the art understand how to display and express any nucleic acid or protein sequence in any of the variety of ways that exist, each of which is considered herein disclosed. Specifically contemplated herein is the display of these sequences on computer readable mediums, such as, commercially available floppy disks, tapes, chips, hard drives, compact disks, and video disks, or other computer readable mediums. Also

disclosed are the binary code representations of the disclosed sequences. Those of skill in the art understand what computer readable mediums. Thus, computer readable mediums on which the nucleic acids or protein sequences are recorded, stored, or saved.

228. Disclosed are computer readable mediums comprising the sequences and information regarding the sequences set forth herein. Also disclosed are computer readable mediums comprising the sequences and information regarding the sequences set forth herein wherein the sequences do not include SEQ ID Nos: 37, 38, 39, 40, 41, and 42.

9. Compositions identified by screening with disclosed compositions

a) Computer assisted drug design

229. The disclosed compositions can be used as targets for any molecular modeling technique to identify either the structure of the disclosed compositions or to identify potential or actual molecules, such as small molecules, which interact in a desired way with the disclosed compositions. The nucleic acids, peptides, and related molecules disclosed herein can be used as targets in any molecular modeling program or approach.]

230. It is understood that when using the disclosed compositions in modeling techniques, molecules, such as macromolecular molecules, will be identified that have particular desired properties such as inhibition or stimulation or the target molecule's function. The molecules identified and isolated when using the disclosed compositions, such as CR2, DAF, CD59, CR1, MCP, Crry, IgG1, IgM, IgG3, CVF, CR2-DAF, DAF-CR2, CR2-CD59, CD59-CR2, CR2-CR1, CR1-CR2, CR2-MCP, MCP-CR2, CR2-Crry, Crry-CR2, CR2-IgG1 Fc (human), CR2-IgM Fc, CR2-IgG3 Fc (murine), or CR2-CVF are also disclosed. Thus, the products produced using the molecular modeling approaches that involve the disclosed compositions, such as, CR2, DAF, CD59, CR1, MCP, Crry, IgG1, IgM, IgG3, CVF, CR2-DAF, DAF-CR2, CR2-CD59, CD59-CR2, CR2-CR1, CR1-CR2, CR2-MCP, MCP-CR2, CR2-Crry, Crry-CR2, CR2-IgG1 Fc (human), CR2-IgM Fc, CR2-IgG3 Fc (murine), or CR2-CVF, are also considered herein disclosed.

231. Thus, one way to isolate molecules that bind a molecule of choice is through rational design. This is achieved through structural information and computer modeling. Computer modeling technology allows visualization of the three-dimensional atomic structure of a selected molecule and the rational design of new compounds that will interact with the molecule. The three-dimensional construct typically depends on data from x-ray crystallographic analyses or NMR imaging of the selected molecule. The molecular dynamics require force field data. The computer graphics systems enable prediction of how a new compound will link to the target molecule and allow experimental manipulation of the structures of the compound and target molecule to perfect binding specificity. Prediction of what the molecule-compound interaction will be when small changes are made in one or both requires molecular mechanics software and computationally intensive computers, usually coupled with user-friendly, menu-driven interfaces between the molecular design program and the user.

232. Examples of molecular modeling systems are the CHARMm and QUANTA programs, Polygen Corporation, Waltham, MA. CHARMm performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modeling and analysis of molecular structure. QUANTA allows interactive construction, modification, visualization, and analysis of the behavior of molecules with each other.

233. A number of articles review computer modeling of drugs interactive with specific proteins, such as Rotivinen, et al., 1988 *Acta Pharmaceutica Fennica* 97, 159-166; Ripka, *New Scientist* 54-57 (June 16, 1988); McKinaly and Rossmann, 1989 *Annu. Rev. Pharmacol._Toxiciol.* 29, 111-122; Perry and Davies, <u>QSAR: Quantitative Structure-Activity Relationships in Drug Design</u> pp. 189-193 (Alan R. Liss, Inc. 1989); Lewis and Dean, 1989 *Proc. R. Soc. Lond.* 236, 125-140 and 141-162; and, with respect to a model enzyme for nucleic acid components, Askew, et al., 1989 *J. Am. Chem. Soc.* 111, 1082-1090. Other computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc., Pasadena, CA., Allelix, Inc, Mississauga, Ontario, Canada, and Hypercube, Inc., Cambridge, Ontario.

Although these are primarily designed for application to drugs specific to particular proteins, they can be adapted to design of molecules specifically interacting with specific regions of DNA or RNA, once that region is identified.

234. Although described above with reference to design and generation of compounds which could alter binding, one could also screen libraries of known compounds, including natural products or synthetic chemicals, and biologically active materials, including proteins, for compounds which alter substrate binding or enzymatic activity.

10. Kits

235. Disclosed herein are kits that are drawn to reagents that can be used in practicing the methods disclosed herein. The kits can include any reagent or combination of reagent discussed herein or that would be understood to be required or beneficial in the practice of the disclosed methods. For example, the kits could include primers to perform the amplification reactions discussed in certain embodiments of the methods, as well as the buffers and enzymes required to use the primers as intended. For example, disclosed is a kit for assessing a subject's risk for cancer, asthma, systemic lupus erythematosus, rheumatoid arthritis, reactive arthritis, spndyarthritis, systemic vasculitis, insulin dependent diabetes mellitus, multiple sclerosis, experimental allergic encephalomyelitis, Sjögren's syndrome, graft versus host disease, inflammatory bowel disease including Crohn's disease, ulcerative colitis, Ischemia reperfusion injury, myocardial infarction, alzheimer's disease, transplant rejection (allogeneic and xenogeneic), thermal trauma, any immune complex-induced inflammation, glomerulonephritis, myasthenia gravis, multiple sclerosis, cerebral lupus, Guillain-Barre syndrome, vasculitis, systemic sclerosis, anaphlaxis, catheter reactions, atheroma, infertility, thyroiditis, ARDS, post-bypass syndrome, hemodialysis, juvenile rheumatoid, Behcets syndrome, hemolytic anemia, pemphigus, bullous pemphigoid, stroke, atherosclerosis, and scleroderma.

11. Compositions with similar funtions

236. It is understood that the compositions disclosed herein have certain functions, such as modulating complement acitvity or binding CR2, CR3, or C3b.

Disclosed herein are certain structural requirements for performing the disclosed functions, and it is understood that there are a variety of structures which can perform the same function which are related to the disclosed structures, and that these structures will ultimately achieve the same result, for example stimulation or inhibition complement activity.

E. Methods of making the compositions

237. The compositions disclosed herein and the compositions necessary to perform the disclosed methods can be made using any method known to those of skill in the art for that particular reagent or compound unless otherwise specifically noted.

238. Disclosed are methods of making a composition comprising a construct, wherein the construct comprises CR2 and a modulator of complement. Also disclosed are methods of making a composition, wherein the composition is the composition of the invention.

1. Peptide synthesis

239. One method of producing the disclosed proteins, such as SEQ ID NO: 6, is to link two or more peptides or polypeptides together by protein chemistry techniques. For example, peptides or polypeptides can be chemically synthesized using currently available laboratory equipment using either Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (*tert* -butyloxycarbonoyl) chemistry. (Applied Biosystems, Inc., Foster City, CA). One skilled in the art can readily appreciate that a peptide or polypeptide corresponding to the disclosed proteins, for example, can be synthesized by standard chemical reactions. For example, a peptide or polypeptide can be synthesized and not cleaved from its synthesis resin whereas the other fragment of a peptide or protein can be synthesized and subsequently cleaved from the resin, thereby exposing a terminal group which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively, to form an antibody, or fragment thereof. (Grant GA (1992) Synthetic Peptides: A User Guide. W.H. Freeman and Co., N.Y. (1992); Bodansky M and Trost B., Ed. (1993) Principles of Peptide Synthesis. Springer-Verlag Inc., NY (which is herein incorporated by reference at least

for material related to peptide synthesis). Alternatively, the peptide or polypeptide is independently synthesized *in vivo* as described herein. Once isolated, these independent peptides or polypeptides can be linked to form a peptide or fragment thereof via similar peptide condensation reactions.

240. For example, enzymatic ligation of cloned or synthetic peptide segments allow relatively short peptide fragments to be joined to produce larger peptide fragments, polypeptides or whole protein domains (Abrahmsen L et al., Biochemistry, 30:4151 (1991)). Alternatively, native chemical ligation of synthetic peptides can be utilized to synthetically construct large peptides or polypeptides from shorter peptide fragments. This method consists of a two step chemical reaction (Dawson et al. Synthesis of Proteins by Native Chemical Ligation. Science, 266:776-779 (1994)). The first step is the chemoselective reaction of an unprotected synthetic peptide--thioester with another unprotected peptide segment containing an amino-terminal Cys residue to give a thioester-linked intermediate as the initial covalent product. Without a change in the reaction conditions, this intermediate undergoes spontaneous, rapid intramolecular reaction to form a native peptide bond at the ligation site (Baggiolini M et al. (1992) FEBS Lett. 307:97-101; Clark-Lewis I et al., J.Biol.Chem., 269:16075 (1994); Clark-Lewis I et al., Biochemistry, 30:3128 (1991); Rajarathnam K et al., Biochemistry 33:6623-30 (1994)).

241. Alternatively, unprotected peptide segments are chemically linked where the bond formed between the peptide segments as a result of the chemical ligation is an unnatural (non-peptide) bond (Schnolzer, M et al. Science, 256:221 (1992)). This technique has been used to synthesize analogs of protein domains as well as large amounts of relatively pure proteins with full biological activity (deLisle Milton RC et al., Techniques in Protein Chemistry IV. Academic Press, New York, pp. 257-267 (1992)).

2. Process for making the compositions

242. Disclosed are processes for making the compositions as well as making the intermediates leading to the compositions. For example, disclosed are nucleic acids in SEQ ID NOs: 5. There are a variety of methods that can be used for making these

compositions, such as synthetic chemical methods and standard molecular biology methods. It is understood that the methods of making these and the other disclosed compositions are specifically disclosed.

243. Disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a nucleic acid comprising the sequence set forth in SEQ ID NO: 25 and a sequence controlling the expression of the nucleic acid.

244. Also disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a nucleic acid molecule comprising a sequence having 80% identity to a sequence set forth in SEQ ID NO: 25, and a sequence controlling the expression of the nucleic acid.

245. Disclosed are animals produced by the process of transfecting a cell within the animal with any of the nucleic acid molecules disclosed herein. Disclosed are animals produced by the process of transfecting a cell within the animal any of the nucleic acid molecules disclosed herein, wherein the animal is a mammal. Also disclosed are animals produced by the process of transfecting a cell within the animal any of the nucleic acid molecules disclosed herein, wherein the mammal is mouse, rat, rabbit, cow, sheep, pig, or primate.

246. Also disclose are animals produced by the process of adding to the animal any of the cells disclosed herein.

247. Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in the sentence in which the reference is relied upon.

248. It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as

exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

F. Examples

249. The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the Dr. Tomlinson regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

1. Example 1: Complement receptor 2 (CR2)-mediated targeting of complement inhibitors to sites of complement activation

a) Methods

(1) Cell lines and DNA.

250. All DNA manipulations were carried out in the mammalian expression vector PBM, derived from p118-mIgG1 (30) by deletion of mouse IgG1 Fc coding region. Chinese hamster ovary (CHO) cells were used for protein expression and were maintained in Dulbecco's modified Eagle's medium (DMEM) (GIBCO Invitrogen Corp, Carlsbad, CA) supplemented with 10% FCS. Stably transfected CHO cell clones were cultivated in the presence of G418, and for recombinant protein expression cells were cultured in suspension in CHO-S-SFM II without FCS (GIBCO). U937 cells were cultured in RPMI (GIBCO), 10% FCS.

(2) Antibodies, reagents and serum.

251. Rabbit antiserum to CHO cell membrane, purified human DAF and CD59 was prepared by standard techniques (31). Mouse anti-DAF mAb 1H4 (32), rat anti-CD59 mAb YTH53.1 (33) and mouse anti human CR2 mAb 171 (binds to SCR 1-2) (34) are described. Anti-sheep erythrocyte IgM was from Research Diagnostic Inc.

(Flanders, NJ). All secondary antibodies were purchased from Sigma (St.Louis, MO). Purified recombinant sCD59 was a gift from Dr. B. P. Morgan (University of Wales, Cardiff, UK). C6-depleted human serum was purchased from Quidel (San Diego, CA) and normal human serum (NHS) was obtained from the blood of healthy volunteers in the laboratory.

(3) Construction of expression plasmids and protein expression.

252. The recombinant fusion proteins and soluble complement inhibitors prepared are depicted in Figure 1. cDNA constructs were prepared by joining the CR2 sequence encoding the 4 N-terminal SCR units (residues 1-250 of mature protein, Swissprot accession no. P20023) to sequences encoding extracellular regions of DAF or CD59. The complement inhibitor sequences used encoded residues 1-249 of mature DAF protein sequence (Swissprot accession no. P08174) and residues 1-77 of mature CD59 protein sequence (Swissprot accession no. P13987). To join CR2 to complement inhibitor sequences, linking sequences encoding $SS(GGGGS)_3$ and $(GGGS)_2$ were used for fusion proteins containing CR2 at the C-terminus and N-terminus, respectively. Gene constructs were prepared by standard PCR methodology (35). All cloning steps were performed in the PBM vector that was also used for protein expression (30). For expression, plasmids were transfected into CHO cells using lipofectamine according to manufacturer's instructions (GIBCO). Stably transfected clones were selected by limiting dilution as described (30) and protein expression of clones quantitated by ELISA.

(4) ELISA and protein assays.

253. Detection of recombinant proteins and determination of relative protein concentration in culture supernatants was achieved using a standard ELISA technique (31). Depending on which type of recombinant protein was being assayed, the capture antibody was either anti-DAF mAb 1H4 or anti-CD59 mAb YTH53.1. Primary detection antibodies were either anti-DAF or anti-CD59 rabbit polyclonal antibody. In some ELISAs, anti-CR2 mAb A-3 was also used as primary detection antibody, and although less sensitive, similar data was obtained. The protein concentration of

recombinant proteins was determined either by UV absorbance or by using a BCA protein assay kit (Pierce Chemical Company, Rockford Ill).

(5) Protein purification.

254. Recombinant proteins were purified from culture supernatant by affinity chromatography. Affinity columns were prepared by coupling either anti-DAF 1H4 mAb or anti-CD59 YTH53.1 mAb to HiTrap NHS-activated affinity columns (Pharmacia Biotech, New Jersey, USA) as described by the manufacturer. Culture supernatants containing recombinant proteins were adjusted to pH 8.0 and applied to affinity columns at a flow rate of 0.5 ml/min. The column was washed with 6 to 8 column volumes of PBS, and recombinant proteins eluted with 2 to 3 column volumes of 0.1 M glycine, pH 2.4. The fractions containing fusion protein were collected into tubes containing 1 M Tris buffer,pH 8.0 and dialyzed against PBS.

(6) SDS-PAGE and Western blotting.

255. Purified recombinant proteins were separated in SDS-PAGE 10% acrylamide gels (Bio-Rad Life Science, Hercules, CA) under nonreducing conditions. Gels were stained with Coomassie blue. For Western blotting, standard procedures were followed (31). Briefly, separated proteins were transferred to a polyvinylidene fluoride membrane, and the transferred proteins detected by means of either anti-DAF mAb 1H4 or anti-CD59 mAb YTH53.1. Membranes were developed with ECL detection kit (Amersham Biosciences, Piscataway, NJ). CR2-CD59 was also analyzed by SDS-PAGE following glycanase treatment. CR2-CD59 (2 mg) was heated at 95 °C for 3 min in 15 mM sodium phosphate buffer (pH 7.5) containing 0.1% SDS, 10 mM 2-mercaptoethanol and 5 mM EDTA. After cooling, CR2-CD59 was incubated with 3 U of Flavobacterium meningosepticum N-glycanase (EC 3.5.1.52, Sigma) for 20 h at 37 oC in the presence of 1% Nonidet P40 and 0.3 mM PMSF.

(7) Flow cytometry.

256. Binding of recombinant fusion proteins to C3-opsonized cells was determined by flow cytometry. CHO cells were incubated in 10% anti-CHO antiserum (30 min/4°C), washed and incubated in 10% C6-depleted NHS (45 min/37°C). The C3 opsonized cells were then washed and incubated with 1 μM recombinant protein (60

min/4°C). After washing, cells were incubated with 10 μg/ml of either anti-DAF mAb 1H4 or anti-CD59 mAb YTH53.1 as appropriate (30 min/4°C), followed by FITC-conjugated secondary antibody (1:100, 30 min/4°C). Cells were then washed, fixed with 2% paraformaldehyde in PBS, and analyzed using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). All incubations and washes were performed in DMEM.

(8) Analysis of CR2 fusion protein binding to C3 ligand.

257. Kinetic analysis of the interaction of the CR2 fusion proteins with C3dg-biotin was performed using surface plasmon resonance (SPR) measurements made on a BIAcore 3000 instrument. Human C3dg-biotin, prepared as described (36), was bound to the surface of BIAcore streptavidin (SA) sensor chips by injecting C3dg-biotin at 50 μg/ml over the surface of one flow cell of the chip at 2 μl/minute for 20 minutes. The flow buffer was 0.5X PBS + 0.05% Tween 20. The SPR signal from captured C3dg generated BIAcore response units ranging from 250-500. Control streptavidin-coated flow cells were run in the absence of protein. Binding was evaluated over a range of CR2 fusion protein concentrations (15.6 -500 nM) in 0.5X PBS, 0.05% Tween 20 at 25 °C at a flow rate of 25 μl/minute. CR2 fusion protein samples were injected in 50 μl aliquots using the kinject command. Association of the fusion proteins with the ligand was monitored for 120 seconds, after which the complex was allowed to dissociate in the presence of buffer only for an additional 120 seconds. The binding surface was regenerated between analyses of different fusion protein concentrations by a 10 second pulse of 200 mM sodium carbonate (pH 9.5) at 50 μl/min. Binding of CR2 fusion protein fragments to C3d-immobilized flow cells was corrected for binding to control flow cells. Binding data were fitted to a 1:1 Langmuir binding model using BIAevaluation Version 3.1 software (BIAcore) and evaluated for best fit by low residual and $\chi 2$ values. The kinetic dissociation profiles obtained were used to calculate on and off-rates (k_a and k_d) and affinity constants (K_D) using the BIAevaluation Version 3.1 program. Between experiments, the streptavidin surface was regenerated with a 60-s pulse of 50 mM sodium hydroxide (pH 9.5) at 50 μl/minute, and C3dg-biotin was reapplied as described above.

(9) Complement lysis assays.

258. CHO cells at 60%–80% confluence were detached with versene (GIBCO), washed twice, and resuspended to 10^6/ml in DMEM. Cells were sensitized to complement by adding 10% rabbit anti-CHO cell membrane antiserum to cells (30 min/4°C). Antiserum was then removed and cells resuspended in NHS diluted in DMEM. Final assay volumes were either 50 or 100 µl. After 45 min at 37°C, cell viability was determined either by trypan blue exclusion (both live and dead cells counted) or ^{51}Cr release (37). Both assays gave similar results. To assay complement inhibitory activity of recombinant proteins, the proteins were diluted in DMEM and added to NHS before addition to CHO cells. A final concentration of 10% NHS was used which resulted in approximately 90% lysis of unprotected antibody sensitized CHO cells. Inhibition of complement-mediated hemolysis was determined using antibody-sensitized sheep erythrocytes (EA) (Advanced Research Technologies, San Diego, CA). Hemolytic assays were carried out in gelatin veronal buffer (GVB^{++}) (Advanced Research Technologies) in a final volume of 300 µl containing 2.5 x 10^7 EA, NHS at a final dilution of 1/300 and incremental concentrations of fusion protein. Reaction mixtures were incubated at 37°C for 60min and reactions were stopped by addition of 300 µl PBS containing 10mM EDTA. Cells were removed by centrifugation and cell lysis assayed by spectrophotometric quantitation of hemoglobin in the supernatant at 413nm.

(10) Adhesion of U937 cells to erythrocytes.

259. Assays of CR3-dependent adhesion to C3-opsonized erythrocytes were performed essentially as described (38). Briefly, fresh sheep erythrocytes (SRBC) were sensitized with a pre-determined sub-agglutinating amount of rabbit anti-SRBC IgM for 30 min at 37°C in GVB (Advanced Research Technologies). After washing twice, C3b-opsonized SRBC were prepared by incubating IgM-sensitized SRBC with an equal volume of a 1:2 dilution of C6-deficient human serum in GVB (120 min/37°C). Cells were washed twice and pellets resuspended in GVB. The majority of C3 bound to erythrocytes following this treatment is in the form of iC3b or C3d degredation products (CR2 ligands) due to the short half life of C3b in serum. U937 cells (4 x 10^5

cells in 200μl) were added to 50μl of C3 opsonized SRBC (2×10^6 cells) and the mixture centrifuged (4min/40 x g) and left at room temperature for 90 min. Cells were then examined by phase contrast microscopy and number of U937 cells adherent to erythrocytes determined. At least 100 erythrocytes were scored per sample, and average number of U937 cells bound per erythrocyte calculated. Triplicate determinations were made for each experiment performed. In some experiments, U937 cells were cultured for 3 days in the presence of 50 ng/ml phorbol myristate acetate (PMA) before harvest, a treatment that results in upregulation of CR3 (39, 40). Cells incubated with IgM-coated SRBC alone, or SRBC incubated directly with C6-deficient human serum were used as controls.

(11) Biodistribution studies.

260. Standard procedures for determining tissue distribution of injected radiolabeled proteins were followed (41, 42). Briefly, 1.7 μg of ^{125}I-labeled CR2-DAF (4.20×10^6 cpm/mg) or sDAF (4.84×10^6 cpm/mg) were injected into the tail vein of 34 week old female NZB/NZW F1 mice (Jackson Labs, Bar Harbor, ME). After 24 h, a blood sample was taken and major organs were removed, shredded and washed in PBS containing 10 mM EDTA, weighed and counted. Targeting specificity was evaluated as percent injected dose per gram tissue. Proteins were iodinated using iodogen method according to manufacturers instructions (Pierce Chemical Co.).

(12) Immunofluorescence microscopy.

261. CR2-DAF or sDAF (270 μg) was injected into the tail vein of 24-week-old MRL/lpr mice. Twenty-four hours later, kidneys were removed and snap frozen. Cryostat sections (5 μm) prepared from frozen kidneys were fixed in acetone and processed for indirect immunofluorescence microscopy. An equimolar mixture of mouse antihuman DAF 1A10 and 1H6 mAbs were used as primary detection antibodies (final concentration, 10 μg/ml) with an anti-mouse IgG Fc-specific FITC-conjugated secondary antibody (F4143, Sigma-Aldrich). Standard procedures were followed (49, incorporated herein by reference for its teaching of antibody staining techniques), except that to reduce background staining, most likely caused by deposited immune complexes in the mouse kidney, the secondary FITC-labeled antibody was diluted

1:800 (10 times the recommended dilution). Digital images were acquired and optimized with Adobe Photoshop using identical settings.

b) Results

(1) Construct design, expression and purification.

262. Recombinant fusion proteins contained the four N-terminal SCR units of human CR2 linked to either the N or C terminus of soluble forms of human CD59 or DAF (constructs depicted in Figure 1). Recombinant proteins were purified from the culture supernatant of stably transfected CHO cell clones with yields of between 100-200 μg/l. Analysis of purified recombinant proteins by SDS-PAGE and Western blot revealed proteins within expected molecular weight range (Figure 2), and except for CR2-CD59, all proteins migrated as a single band. The two bands seen for CR2-CD59 were due to differences in glycosylation, since CR2-CD59 migrated as a single band following glycanase treatment.

(2) Targeting of fusion proteins to complement opsonized cells.

263. C3 ligand for CR2 was deposited on CHO cells by incubation of CHO cells with complement activating antibody and C6-depleted serum (to prevent MAC formation and cell lysis). All CR2-containing fusion proteins, but not sCD59 or sDAF, bound to C3-coated CHO cells (Figure 3).

(3) Kinetic analysis of interaction between fusion proteins and C3dg ligand.

264. A comparison of the affinity of the different recombinant fusion proteins for the CR2 ligand C3dg was determined by surface plasmon resonance measurements. The experiments were performed by passing varying concentrations of the fusion proteins over Biacore streptavidin chips containing captured C3dg-biotin (approximately 2000 response units). Kinetic analysis of the data showed the best fit to a 1:1 (Langmuir) binding interaction model using global fitting parameters (Figure 4). Both of the fusion proteins with CR2 at the N-terminus (CR2-DAF and CR2-CD59) showed similar binding profiles, with a fast association and a fast dissociation rate. In contrast, binding of fusion proteins with CR2 at the C-terminus (DAF-CR2 and CD59-

CR2) showed slow association and dissociation rates (Figure 4, Table 1). The N-terminus CR2 fusion proteins, however, bound with the highest affinity (Table 1). CD59 fusion proteins bound with a higher affinity than DAF fusion proteins. Soluble DAF and sCD59 did not bind to immobilized C3dg.

(4) Complement inhibitory activity of fusion proteins.

265. Complement inhibitory activity of the targeted and untargeted complement inhibitors was analyzed by measuring their effect on complement-mediated lysis of both CHO cells and erythrocytes. In these experiments, antibody sensitized cells and recombinant proteins were incubated in human serum at a concentration that resulted in 90-100% lysis of unprotected cells. For both cell types, the targeted complement inhibitors were significantly more effective than their respective untargeted proteins at inhibiting complement-mediated lysis. Targeted DAF proteins were more effective inhibitors than targeted CD59 (Figures 5 and 6). Fusion proteins containing CR2 linked to the N-terminus of either DAF and CD59 were more effective inhibitors than C-terminal CR2 fusion proteins. The most potent inhibitor of complement lysis was CR2-DAF, requiring a concentration of 18 nM for 50% inhibition of CHO cell lysis. In contrast, untargeted sDAF required a concentration of 375 nM for 50% inhibition of CHO cell lysis, a 20-fold difference (Figure 5a). sCD59 was a particularly poor inhibitor of complement and provided only 25% protection from CHO cell lysis at 500 nM, the highest concentration tested. CR2-CD59, however, provided 50% inhibition of CHO cell lysis at 102 nM and was more effective than untargeted sDAF (Figure 6a). Table 4 compares the inhibitory activities of the different recombinant complement inhibitors. The higher complement inhibitory activity of the N-terminus CR2 fusion proteins correlated with the higher affinity these proteins exhibited for C3dg ligand (Table 3).

266. There were some differences between the relative effectiveness of the complement inhibitors at protecting CHO cells and erythrocytes form complement-mediated lysis. This was particularly true for the DAF inhibitors; sDAF was significantly more effective at protecting erythrocytes than CHO cells from complement, although targeted DAF was still more effective. There was also little

difference in the inhibitory activity of CR2-DAF and DAF-CR2 when erythrocytes were the target cells for complement lysis.

(5) Effect of CR2-fusion proteins on cell adhesion.

267. Complement receptor 3 is a leukocyte receptor involved in endothelial adhesion and diapedesis and the activation of cell cytolytic mechanisms (phagocytosis and degranulation). Since CR2 and CR3 share the same iC3b complement ligand, it was determined whether CR2 fusion proteins interfered with CR3-mediated cell binding. For these experiments U937, a well characterized promonocytic cell line ($CR2^-$, $CR3^+$) that binds to iC3b coated erythrocytes in a CR3-dependent mechanism, was used (40). All of the CR2 fusion proteins, but not sDAF or sCD59, significantly inhibited the binding of U937 cells to C3 opsonized sheep erythrocytes ($P<0.01$). Each CR2 fusion protein inhibited U937 binding to a similar extent at a concentration of 500 nM (Figure 7). Similar data was obtained in an experiment using U937 cells that were stimulated with PMA, a treatment that results in upregulation of CR3 (39, 40). For complement opsonization of erythrocytes, IgM was used to activate complement since IgG deposited on the erythrocytes would engage Fcγ receptors expressed on U937 cells. U937 cells also express CR4 (p150,95, CD11c/CD18), a third receptor sharing the iC3b ligand. However, binding of U937 cells to C3-opsonized erythrocytes is CR4-independent, probably due to the association of CR4 with the cytoskeleton and its immobility in the membrane (40).

(6) Targeting of CR2-DAF to the kidneys of nephritic mice.

268. To determine whether a CR2 fusion protein will target a site of complement activation and disease in vivo, a biodistribution study of CR2-DAF and sDAF in female NZB/W F1 mice was performed. NZB/W F1 mice develop a spontaneous autoimmune disease that is very similar to human systemic lupus erythematosus (SLE), with the production of autoantibodies and the development of severe immune complex–mediated glomerulonephritis that is associated with complement deposition from 26 to 28 weeks of age (4, 52). Biodistribution of $[^{125}I]$CR2-DAF and $[^{125}I]$sDAF in 34-week-old NZB/W F1 mice was determined at 24

hours and 48 hours after injection. Twenty-four hours after tail-vein injection of [^{125}I]CR2-DAF, a significantly higher proportion of radioactivity was localized to the kidney than to the other organs that were examined (Figure 25a). At 48 hours after injection of [^{125}I]CR2-DAF, there was a similar level of radioactivity in the kidney as at 24 hours, but radioactivity in the liver and spleen was increased and blood radioactivity decreased (Figure 25b). The liver and spleen are sites of immune complex clearance and likely account for increased targeting of [^{125}I]CR2-DAF to these organs at the later time point. [^{125}I]sDAF showed no preferential binding in the kidney or any other organ (Figure 25, a and b). In 8-weekold prenephritic NZB/W F1 mice, there was no evidence of [^{125}I]CR2-DAF targeting to the kidney (Figure 25c). Of further interest, [^{125}I]sDAF was cleared much more rapidly from the circulation than [^{125}I]CR2-DAF, suggesting that the CR2 moiety is functioning to prolong the circulatory half-life of the fusion protein. However, the level of [^{125}I]CR2-DAF in the blood of younger mice at 24 hours was about half that recorded in the older mice, and the long circulatory half-life of [^{125}I]CR2-DAF may be a consequence, at least in part, of it binding to circulating immune complexes.

269. Targeting of CR2-DAF to complement deposited in the kidney was also examined in another murine model of SLE by direct examination of kidney sections. Similar to female NZB/W F1 mice, MRL/lpr mice develop severe proliferative glomerulonephritis with the deposition of complement in association with glomerular immune deposits by 24 weeks of age (53, incorporated herein for its teaching of this mouse model). CR2-DAF and sDAF were injected into the tail vein of 24-week-old MRL/lpr mice, and kidney sections were analyzed 24 hours later for human DAF immunoreactivity by fluorescence microscopy. Kidney sections from a mouse injected with CR2-DAF displayed a high level of DAF staining, with preferential localization in glomeruli in a pattern identical to that seen for immune complexes. No DAF staining was evident in glomeruli from a mouse injected with sDAF (Figure 26).

c) Conclusions

270. This study describes the generation and characterization of soluble human DAF and CD59 containing proteins that are targeted to a site of complement

activation. The targeted proteins were significantly more potent at inhibiting complement than their untargeted counterparts. Targeting of CD59 and DAF was achieved by linking the inhibitors to a fragment of human CR2 that binds complement C3 activation products. The C3 ligands for CR2 are relatively long lived and are covalently bound, often in large quantities, at sites of complement activation. Thus, CR2-mediated targeting of complement inhibition isof therapeutic benefit for numerous complement-associated diseases or disease states. Consistent with this hypothesis, CR2-DAF was shown to target to the kidneys of nephritic NZB/W F1 mice. These mice produce autoantibodies with consequent formation and deposition of immune complexes in the kidney resulting in complement activation and deposition (2, 43). Human CR2 binds human and mouse C3 ligands with similar affinities (44), and the biodistribution studies establish that a CR2-fusion protein retains targeting function *in vivo*. This study establishes the feasibility of this approach for human complement inhibition. The targeting approach can also be effective for other inhibitors of complement activation such as soluble CR1, which is in clinical trials and is a more potent inhibitor of complement than DAF in vitro (9).

271. The relative affinities for C3dg of the different CR2 fusion proteins is reminiscent of the affinities of SCR 1-2 of CR2 and SCR 1-15 of CR2for C3dg. The KD values for CR2 SCR1-2 and CR2 SCR 1-15 interactions with C3dg were similar, but CR2 SCR 1-2 associated and dissociated much faster, indicating a contribution of the additional SCR domains to overall affinity (36). Analysis of the solution structure of another SCR containing protein, factor H, indicated that SCR domains are folded back on themselves and interactions between SCR domains can modulate C3 ligand binding characteristics (45). Conformational variability between SCR domains is predicted to result from different (native) linker lengths, with longer linkers providing greater conformational flexibility. In this context, the CR2 and DAF SCR domains are linked with a relatively long ser-gly linker, and this can permit the fusion partners to fold back on one another resulting in SCR-SCR interactions that can modulate CR2 binding affinity.

272. Complement-mediated lysis assays were performed using antibody sensitized CHO cells or sheep erythrocytes as targets. There were marked differences in the relative activities of some of the complement inhibitors at protecting the different cells from complement-mediated lysis. sDAF, DAF-CR2, and CD59-CR2 were significantly more effective at protecting sheep erythrocytes than CHO cells from complement-mediated lysis. Unlike rythrocytes, complement-mediated lysis of nucleated cells is not due entirely to colloid osmotic deregulation, and the deposition of multiple MACs in the plasma membrane is required (46-48). The majority of previous studies investigating the inhibitory activity of soluble (untargeted) complement inhibitors have been performed using erythrocytes as target cells for complement mediated lysis. However, CHO cells likely represent a more physiologically relevant target for in vitro experiments.

273. Different mechanisms of complement-mediated damage are implicated in different disease conditions and different diseases can benefit from inhibition strategies acting at different points in the pathway. For example, if applicable for the disease, a particular benefit of blocking complement at a late step in the pathway would be that host defense functions and immune homeostasis mechanisms of complement would remain intact. Thus, a CD59-based inhibitor would provide advantages over inhibitors of complement activation in diseases in which the terminal cytolytic pathway is primarily implicated in pathogenesis. Soluble CD59 is unlikely to have therapeutic benefit due to its very poor activity in vitro, but it was shown herein that CR2-mediated targeting of CD59 significantly increased its complement inhibitory activity. In fact, CR2-CD59 was more effective at inhibiting complement-mediated lysis than sDAF, and sDAF has shown therapeutic efficacy in vivo (8). Rodent analogues of CR2-CD59 can also be a useful tools for dissecting the relative roles of early complement activation products vs. MAC formation in disease pathogenesis. The relative contributions of the different complement activation products to tissue injury in many disease states is poorly understood and controversial.

274. The CR2 fusion proteins inhibited the binding of U937 cells to C3-opsonized erythrocytes. CR2 and CR3 both bind iC3b, and this data indicates that CR2

fusion proteins act as CR3 antagonists since U937 binding to C3-opsonized erythrocytes is CR3-dependent (40). As an adhesion molecule, CR3 mediates endothelial adhesion and diapedesis at sites of inflammation via its high affinity interaction with intercellular adhesion molecule-1 (ICAM-1). As a complement receptor, CR3 promotes and enhances phagocytosis and degranulation via its interaction with iC3b. Both ICAM-1 and iC3b bind to overlapping epitopes on CR3 (see Ross review). CR3 can thus be an important determinant in promoting cell-mediated tissue damage at sites of inflammation, and antibodies that block CR3 have shown effectiveness in several inflammatory conditions (see Ross review). The antagonistic effect of CR2 on CR3 binding therefore indicates a second anti-inflammatory mechanism of action of the CR2-complement inhibitor fusion proteins that act synergistically with complement inhibition.

275. Targeting complement inhibitors to sites of complement activation and disease can considerably enhance their efficacy. Indeed, for disease states that would benefit from CD59-based therapy, the targeting of CD59 to the site of complement activation will be a requirement. An advantage of CR2-mediated targeting over other targeting approaches, such as antibody-mediated targeting, is that the CR2 moiety targets any accessible site of complement activation and has broad therapeuticapplication. CR2 fusion proteins can also act as CR3 antagonists, and this can represent a second important therapeutic benefit. Human CR2-complement inhibitor fusion proteins are also much less likely to be immunogenic than recombinant inhibitors containing antibody variable regions. The predicted ability of targeted inhibitors of complement activation to provide an effective local concentration with low levels of systemic inhibition also diminishes the possibility of compromising host defense mechanisms, particularly with long term systemic complement inhibition (this is a less important consideration for CD59-based inhibitors). CR2-tageted inhibitors can also target infectious agents that activate complement.

2. Example 2: Targeted complement inhibition and activation

a) Complement inhibitors (inflammation/bioincompatibility)

276. Complement inhibitors hold considerable promise for the therapy of many autoimmune and inflammatory diseases, and disease states associated with bioincompatibility. A safe and effective pharmaceutical inhibitor of complement is not currently available. Research has largely focused on developing soluble inhibitors based on host membrane-bound complement-regulatory proteins. Recombinant forms of soluble CR1, MCP, DAF and Crry have been produced by removal of membrane-linking regions, and all proteins have been shown to be effective at reducing inflammation and complement-mediated tissue damage in various models of disease. Soluble CR1 and an antibody that blocks the function of complement protein C5 are in clinical trials. There are, however, serious questions concerning the clinical use of systemically administered soluble complement inhibitors. Complement plays a crucial role in both innate and adaptive immunity, and the generation of C3b is critical for the opsonization and leukocyte-mediated clearance of many pathogenic microorganisms. In addition, the fluid phase complement activation product C5a has been shown to be important in controlling infection and can be important in the clearance of pathogenic substances from the circulation. Systemic inhibition of complement is therefore likely to have serious consequences for the host regarding its ability to control infection. Complement is also crucial for the effective catabolism of immune complexes, and this is a particularly important consideration in the use of complement inhibitors for the treatment of autoimmune and immune complex diseases.

277. The targeting of complement inhibitors to sites of complement activation and disease can allow a much lower effective serum concentration and significantly reduce the level of systemic complement inhibition. Increased efficacy is an important benefit of targeted complement inhibitors, and targeting can also address the problem of a short half life of soluble recombinant complement inhibitors in the circulation.

278. In addition to the above considerations with regard to the targeting of complement inhibitors, selectively blocking different parts of the complement pathway can allow the generation of beneficial complement activation products, but inhibit the generation of complement activation products involved in disease pathogenesis. For example, inhibitors of complement activation (such as CR1, DAF, Crry) inhibit C3b,

C5a and C5b-9 generation. Antibodies to C5 inhibit C5a and C5b-9 generation. On the other hand, CD59-based inhibitors do not effect C3b and C5a generation, but block only C5b-9 formation (see Figure 8). The terminal complement pathway and C5b-9 generation has been shown to be important in promoting inflammation and is in particular implicated in the progression of some diseases of the kidney (such as immune complex glomerulonephritis). Thus, for certain diseases, a CD59-based inhibitor can inhibit disease pathogenesis without interfering with the generation of early complement activation products that are important for host defense and immune complex clearance. However, soluble CD59 is not an effective inhibitor of complement (unlike inhibitors of activation DAF, CR1, MCP or Crry), and is unlikely to have any clinical application. Cell targeted CD59, however, can represent a viable therapeutic. Data using antibody-mediated targeting of CD59 [Zhang et al., 1999, J.Clin.Invest., 103, 55-61], and the data presented herein with CR2-mediated targeting of CD59 show that CD59 targeted to a cell membrane is significantly more effective than soluble untargeted CD59.

b) Complement activators (cancer)

279. The initial promise of anti-tumor complement activating monoclonal antibodies as cancer immunotherapeutic agents has not been realized. One reason for this is the expression of complement inhibitory proteins on tumor cells (complement inhibitors are often upregulated on tumor cells). Thus, although certain antibodies have been shown to target tumors in humans and to activate complement on the tumor cell surface, the tumor cells resist complement-mediated destruction. There is a large body of evidence from *in vitro* studies indicating an important role for complement inhibitors in tumor resistance to antibody therapy. In addition, reports [Caragine,et al., 2002, Cancer Res, 62, 1110-15; Chenet al., 2000, Cancer Res., 60, 3013-18; Baranyi et al., 1994, Immunology, 82, 522-8] have established that complement inhibitors expressed on the surface of tumor cells *in vivo* have functional consequences with regard to complement deposition and tumorigenesis. Enhancing complement deposition on tumor cells allows more effective immune-mediated clearance of tumor cells and improve prospects for successful immunotherapy using complement-activating anti-tumor

antibodies. Enhanced complement activation overwhelms tumor cell expressed complement inhibitory proteins.

c) RESULTS - 1

(1) Targeted complement inhibitor fusion protein

280. Examples of human fusion proteins that have been expressed, purified and characterized for targeting and assessed for complement inhibitory function in vitro as previously described include the following: CR2-DAF, CR2-CD59, DAF-CR2, and CD59-CR2. The nucleotide sequences and predicted amino acid sequences of mature human fusion proteins are shown in Figures 8-11.

(2) EXPRESSION AND PURIFICATION

281. cDNA plasmid constructs encoding the fusion proteins were transfected into CHO cells and stably expressing clones isolated. Clones expressing highest levels of fusion protein were selected. The selected clones were grown in bioreactors and fusion proteins isolated from culture supernatant by affinity chromatography. Affinity columns were prepared using anti-DAF and anti-CD59 antibodies conjugated to sepharose. Recombinant proteins were analyzed by SDS-PAGE and Western blot (Figure 2).

(3) BINDING OF FUSION PROTEINS TO C3 LIGANDS.

(a) Flow cytometry

282. Flow cytometry experiments were conducted as previously described. All of the CR2 containing fusion proteins bound to C3-coated CHO cells, as analyzed by flow cytometry (Figure 12). sDAF and sCD59 did not bind to C3-coated CHO cells.

(b) ELISA

283. ELISA experiments were conducted as previously described. In ELISA experiments, CR2-containing constructs were added to wells coated with purified C3dg. Binding was detected by means of anti-complement inhibitor antibodies and enzyme-congugated secondary antibodies. All CR2 containing constructs, but not sCD59 and sDAF bound to C3d.

(c) Surface plasmon resonance

284. Biotinylated C3dg (CR2 ligand) was bound to streptavidin coated BIAcore chips and binding kinetics of CR2 containing fusion proteins measured (Figures 13-16). sDAF and sCD59 did not bind to captured C3dg. Fusion proteins with CR2 at N-terminus bound with highest affinity. CD59 containing fusion proteins bound with higher affinity than corresponding DAF containing fusion proteins.

(4) COMPLEMENT INHIBITORY FUNCTION OF FUSION PROTEINS

285. The functional activity of the fusion proteins and soluble untargeted complement inhibitors was analyzed by measuring the effect of the proteins on complement-mediated cell lysis. Assays using Chinese hamster ovary (CHO) cells (Figures 17 and 18)and sheep erythrocytes (E) (Figures 19 and 20) were used.

286. The targeted complement inhibitors provided significantly more protection from complement-mediated than soluble untargeted complement inhibitors. Fusion proteins containing CR2 at the N-terminus were the most effective for both DAF and CD59 containing fusion proteins. N-terminal CR2 fusion proteins also bound C3d with a higher affinity than C-terminal CR2 fusion proteins in BIAcore experiments (see above). CR2-DAF was significantly more effective than CR2-CD59 at providing protection from complement-mediated lysis in these assays. Of note, however, untargeted sCD59 possesses very weak complement inhibitory activity even at high concentration (unlike sDAF), and the targeting of CD59 to the cell surface is a requirement for CD59 function.

287. The relative effectiveness of targeted vs. untargeted complement inhibitors for CHO cells and E is different. However, erythrocytes are lysed by "one hit" (ie. formation of a single MAC causes E lysis), whereas nucleated cells (such as CHO) posses addition resistance mechanisms (such as capping and shedding of MACs) and require deposition of multiple MACs for lysis. These differences likely account for differences in lysis inhibition data, and CHO cells likely represent the more physiologically relevant target for these in vitro experiments.

D) RESULTS - 2

(1) Targeted complement activating fusion/conjugated proteins

288. Human CR2-IgG1 Fc has been expressed and purified and shown to appropriately target C3 opsonized cells in vitro. Expression plasmids containing encoding sequences for human and mouse sCR2 (for conjugation with CVF) and mouse fusion proteins have been prepared. Nucleotide sequence and predicted amino acid sequences of mature human fusion proteins are shown in Figure 21

(2) EXPRESSION AND PURIFICATION

289. cDNA encoding the first 4 SCRs of CR2 was linked to genomic sequence encoding human IgG1 Fc region. Plasmid encoding the fusion protein was transfected into CHO cells and stably expressing clones isolated. Clones expressing highest levels of fusion protein were selected. The selected clone was grown in a bioreactor and fusion proteins isolated from culture supernatant by protein A affinity chromatography. Recombinant protein was analyzed by SDS-PAGE (Figure 22) and Western blot. Protein migrated at expected molecular weight under reducing and nonreducing conditions (CR2-Fc is disulfide linked dimer). A murine plasmid construct encoding CR2-mouse IgG3 has been constructed.

(3) BINDING OF FUSION PROTEINS TO C3 LIGANDS.

(a) Flow cytometry

290. CR2-Fc bound to C3-coated CHO cells, as analyzed by flow cytometry (Figure 23).

(b) ELISA

291. In ELISA experiments, CR2-Fc was added to wells coated with purified C3dg. Binding was detected by means of anti-human Fc antibodies and enzyme-congugated secondary antibodies. CR2-Fc bound to C3d.

(c) Surface plasmon resonance

292. Biotinylated C3dg (CR2 ligand) was bound to streptavidin coated BIAcore chips and binding of CR2-Fc demonstrated (Figure 24).

3. Example 3

a) Antibody targeted complement inhibitors in a rat model of acute tubulointerstitial injury:

293. A panel of well characterized mouse anti-rat kidney monoclonal antibodies was used (49, 50, incorporated herein by reference for their teaching regarding these antibodies and their sequences). The variable region DNA from a total of 5 antibodies was isolated by standard PCR techniques (35, incorporated herein by reference for its teachings regarding PCR). All were successfully cloned and some were expressed as single chain antibodies. All single chain antibodies recognized either a rat kidney epithelial or endothelial cell line in vitro. One of the mAbs, K9/9, binds to a glycoprotein identified on the epithelial cell surface, and has specificity for the glomerular capillary wall and proximal tubules in vivo (49). This antibody was chosen as a targeting vehicle for investigation of targeted Crry- and CD59-mediated complement inhibition in a rat model of acute tubulointerstitial injury. Although the K9/9 mAb was shown to induce glomerular damage in a previous study (49), the antibody was only pathogenic when administered together with Freunds adjuvant. In fact the pathogenic nature of K9/9 mAb (with adjuvant) was not reproduced.

294. There is a link between proteinuria and progressive renal damage and there is data to support the hypothesis that proteinuria itself results in interstitial fibrosis and inflammation. The mechanism by which proteinuria leads to nephrotoxic injury is not known, but there is evidence that complement plays a key role and that the MAC is the principal mediator of tubulointerstitial injury due to proteinuria. (The role of complement and proteinuria in tubulointerstitial injury has been recently reviewed (51, 52)). Previous characterization of K9/9 mAb (see above (49)) suggested that the mAb targets appropriately for an investigation into the therapeutic use of targeted complement inhibitors in a rat model tubulointerstitial injury induced by proteinuria. The availability of an inhibitor that can specifically block MAC formation would allows an assessment of the role of MAC in tubulointerstitial injury under clinically relevant conditions.

295. Plasmid constructs encoding single chain K9/9 antibody linked to rat Crry or rat CD59 were prepared (depicted in fig. 27). Constructs expressing soluble rat Crry (sCrry) and single chain K9/9 (targeting vehicle only) were also prepared. All recombinant proteins were expressed into the culture medium at over 15 mg/liter by Pichia fermentation in a 15 liter New Brunswick fermentor.

296. Recombinant proteins were characterized for targeting and complement inhibitory activity in vitro as described above using a rat epithelial cell line as target cells. Single chain K9/9, K9/9-Crry and K9/9-CD59 specifically bound to rat epithelia cells in vitro. sCrry and K9/9-Crry inhibited complement deposition and lysis, and K9/9-CD59 inhibited complement-mediated lysis. Both targeted complement inhibitors, sCrry and K9/9 single chain Ab were characterized in the rat puromycin aminonucleoside (PAN) nephrosis model (53, incorporated herein by reference for its teaching of sCrry and K9/9) (sCD59 was not evaluated since untargeted CD59 has only very poor complement inhibitory activity (54)). First, to confirm kidney targeting of K9/9 fusion proteins, in vivo binding specificity was determined by biodistribution of iodinated proteins as described above (54, 41). Single chain K9/9 and K9/9 fusion proteins, but not sCrry, specifically targeted to rat kidneys and was detectable at 48 hr after administration (fig. 28). Biodistribution at 24 hr after administration was similar, and there was no radiolabel remaining in the blood at 24 hr.

297. In therapeutic studies, groups of 4 rats received PAN (150 mg/kg) at day 0 and either PBS or complement inhibitor (40 mg/kg) on days 4,7 and 10. Urine (metabolic cages) and blood was collected and animals sacrificed on day 11. PAN treatment significantly impaired renal function as measured by creatinine clearance (fig. 29, second bar from left). There was a slight, but not significant improvement in renal function in rats receiving sCrry therapy. However, creatinine clearance was significantly improved in PAN treated rats receiving either targeted Crry or CD59 therapy ($p<0.01$). There was no significant difference in creatinine clearance between control (non-proteinuric) rats and PAN treated rats receiving either of the targeted inhibitors (fig. 29). As expected, PAN-induced proteinuria was high in all rats whether treated with complement inhibitors or not (table 1). Kidney sections prepared from rats

treated with PAN and receiving no therapy showed dilation of tubular lumina and tubular and epithelial cell degeneration as assessed by loss of brush border (see fig. 30b, also in appendix). Minimal improvement was seen with sCrry therapy (fig. 30d). In contrast tubular dilation and degeneration was significantly suppressed in PAN-treated rats receiving targeted Crry and CD59 (fig. 30c shows K9/9-Crry, but histology was indistinguishable with K9/9-CD59). These data demonstrate therapeutic efficacy of complement inhibition in this model, demonstrate significant benefit of targeted vs untargeted complement inhibiton and directly demonstrate an important role for MAC-mediated damage in tubulointerstial injury induced by proteinuria. sCD59 is not an effective inhibitor and this study demonstrates that appropriately targeted CD59 allows for the specific inhibition of the MAC in vivo.

298. In a separate experiment the circulatory half life of iodinated recombinant proteins was determined as described (54, 41, incorporated herein by reference for the techniques taught therein). The half lives (t1/2) of the proteins were as follows: sCrry: 19 min, K9/9-Crry: 23 min, K9/9-CD59, 29 min, single chain K9/9: 21 min. To determine the effect of the recombinant proteins on systemic complement inhibition, rats were injected with proteins at 40 mg/Kg and blood collected at times corresponding to 1, 3, 5 and 7 x t1/2. Complement inhibitory activity in serum was determined by measuring hemolytic activity (sensitized sheep erythrocytes). As expected, K9/9-CD59 had minimal inhibitory activity in serum (untargeted assay system) (fig. 31). By about 3 hr (7 x t1/2) after the injection of sCrry and K9/9-Crry, there was minimal complement inhibitory activity remaining in serum. The short t1/2 of targeted and untargeted inhibitors, together with biodistribution data and the fact that sCrry is not protective, demonstrate that the kidney-bound complement inhibitors are effective at inhibiting complement locally and for a prolonged period.

299. These data establish the use of targeted complement inhibitors in vivo and demonstrate important benefits of targeted versus untargeted systemic complement inhibition in a model of disease. Although a different targeting vehicle is used in these studies (an antibody fragment), the same principles apply for other targeting vehicles, such as CR2.

4. Example 4

300. Disclosed herein are examples of constructs of the present invention made in accordance with the teaching herein. The terminology used has the following meaning: SCR = short consensus repeats; LP = Leader Peptide. The constructs all have the basic formula of CR2-linker-complement modulator or complement modulator-linker-CR2. Notations in parenthesis indicate details within a particular section of the composition. For example, "(complete)" means that the entire mature protein is used in the construct, whereas "(SCR2-4)" indicates that SCR1 is not part of the construct. It is understood that a linker can be a chemical linker, a natural liker peptide, or amino acid linking sequences (e.g., $(Gly_4Ser)_3$). It is understood that this list is not limiting and only provides examples of some of the constructs disclosed in the present application.

CR2 (complete) – $(Gly_4Ser)_3$--DAF
CR2 (complete) – $(Gly_4Ser)_3$—human CD59
CR2 (complete) – $(Gly_4Ser)_3$--MCP
CR2 (complete) – $(Gly_4Ser)_3$—CR1
CR2 (complete) – $(Gly_4Ser)_3$--Crry
CR2 (complete) – $(Gly_4Ser)_3$—mouse CD59
CR2 (complete) – $(Gly_4Ser)_3$—human IgG1 Fc
CR2 (complete) – $(Gly_4Ser)_3$—human IgM Fc
CR2 (complete) – $(Gly_4Ser)_3$—murine IgG3 Fc
CR2 (complete) – $(Gly_4Ser)_3$—murine IgM Fc
CR2 (complete) – $(Gly_4Ser)_3$--CVF
CR2 (complete) – $(Gly_3Ser)_4$--DAF
CR2 (complete) – $(Gly_3Ser)_4$—human CD59
CR2 (complete) – $(Gly_3Ser)_4$--MCP
CR2 (complete) – $(Gly_3Ser)_4$—CR1
CR2 (complete) – $(Gly_3Ser)_4$--Crry
CR2 (complete) – $(Gly_3Ser)_4$—mouse CD59

CR2 (complete) – $(Gly_3Ser)_4$—human IgG1 Fc

CR2 (complete) – $(Gly_3Ser)_4$—human IgM Fc

CR2 (complete) – $(Gly_3Ser)_4$—murine IgG3 Fc

CR2 (complete) – $(Gly_3Ser)_4$—murine IgM Fc

CR2 (complete) – $(Gly_3Ser)_4$—CVF

CR2 (complete) – $(Gly_4Ser)_3$—DAF (SCRs 2-4)

CR2 (complete) – $(Gly_3Ser)_4$—DAF (SCRs 2-4)

CR2 (complete) – $(Gly_4Ser)_3$—CR1 (LP--SCR1-4—SCR8-11—SCR15-18)

CR2 (complete) – $(Gly_4Ser)_3$—Crry (5 N-terminal SCRs)

CR2 (complete) – VSVFPLE--DAF

CR2 (complete) – VSVFPLE —human CD59

CR2 (complete) – VSVFPLE --MCP

CR2 (complete) – VSVFPLE —CR1

CR2 (complete) – VSVFPLE --Crry

CR2 (complete) – VSVFPLE —mouse CD59

CR2 (complete) – VSVFPLE —human IgG1 Fc

CR2 (complete) – VSVFPLE —human IgM Fc

CR2 (complete) – VSVFPLE —murine IgG3 Fc

CR2 (complete) – VSVFPLE —murine IgM Fc

CR2 (complete) – VSVFPLE —CVF

CR2 (complete) --- *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester --DAF

CR2 (complete) --- *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —human CD59

CR2 (complete) —-*m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester --MCP

CR2 (complete) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —CR1

CR2 (complete) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester --Crry

CR2 (complete) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —mouse CD59

CR2 (complete) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —human IgG1 Fc

CR2 (complete) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —human IgM Fc

CR2 (complete) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —murine IgG3 Fc

CR2 (complete) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —murine IgM Fc

CR2 (complete) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —CVF

CR2 (complete) – bismaleimidohexane --DAF

CR2 (complete) – bismaleimidohexane —human CD59

CR2 (complete) – bismaleimidohexane --MCP

CR2 (complete) – bismaleimidohexane —CR1

CR2 (complete) – bismaleimidohexane --Crry

CR2 (complete) – bismaleimidohexane —mouse CD59

CR2 (complete) – bismaleimidohexane —human IgG1 Fc

CR2 (complete) – bismaleimidohexane —human IgM Fc

CR2 (complete) – bismaleimidohexane —murine IgG3 Fc

CR2 (complete) – bismaleimidohexane —murine IgM Fc

CR2 (complete) – bismaleimidohexane —CVF

CR2 (SCR1-2) – $(Gly_4Ser)_3$--DAF

CR2 (SCR1-2)– $(Gly_4Ser)_3$—human CD59

CR2 (SCR1-2) – $(Gly_4Ser)_3$--MCP

CR2 (SCR1-2) – $(Gly_4Ser)_3$—CR1

CR2 (SCR1-2) – $(Gly_4Ser)_3$--Crry

CR2 (SCR1-2)– $(Gly_4Ser)_3$—mouse CD59

CR2 (SCR1-2) – $(Gly_4Ser)_3$—human IgG1 Fc

CR2 (SCR1-2) – $(Gly_4Ser)_3$—human IgM Fc

CR2 (SCR1-2) – $(Gly_4Ser)_3$—murine IgG3 Fc

CR2 (SCR1-2) – $(Gly_4Ser)_3$—murine IgM Fc
CR2 (SCR1-2) – $(Gly_4Ser)_3$--CVF
CR2 (SCR1-2) – $(Gly_3Ser)_4$--DAF
CR2 (SCR1-2) – $(Gly_3Ser)_4$—human CD59
CR2 (SCR1-2) – $(Gly_3Ser)_4$--MCP
CR2 (SCR1-2) – $(Gly_3Ser)_4$—CR1
CR2 (SCR1-2) – $(Gly_3Ser)_4$--Crry
CR2 (SCR1-2) – $(Gly_3Ser)_4$—mouse CD59
CR2 (SCR1-2) – $(Gly_3Ser)_4$—human IgG1 Fc
CR2 (SCR1-2) – $(Gly_3Ser)_4$—human IgM Fc
CR2 (SCR1-2) – $(Gly_3Ser)_4$—murine IgG3 Fc
CR2 (SCR1-2) – $(Gly_3Ser)_4$—murine IgM Fc
CR2 (SCR1-2) – $(Gly_3Ser)_4$—CVF
CR2 (SCR1-2) – $(Gly_4Ser)_3$—DAF (SCRs 2-4)
CR2 (SCR1-2) – $(Gly_3Ser)_4$—DAF (SCRs 2-4)
CR2 (SCR1-2) – $(Gly_4Ser)_3$—CR1 (LP--SCR1-4—SCR8-11—SCR15-18)
CR2 (SCR1-2) – $(Gly_4Ser)_3$—Crry (5 N-terminal SCRs)
CR2 (SCR1-2) – VSVFPLE--DAF
CR2 (SCR1-2) – VSVFPLE —human CD59
CR2 (SCR1-2) – VSVFPLE --MCP
CR2 (SCR1-2) – VSVFPLE —CR1
CR2 (SCR1-2) – VSVFPLE --Crry
CR2 (SCR1-2) – VSVFPLE —mouse CD59
CR2 (SCR1-2) – VSVFPLE —human IgG1 Fc
CR2 (SCR1-2) – VSVFPLE —human IgM Fc
CR2 (SCR1-2) – VSVFPLE —murine IgG3 Fc
CR2 (SCR1-2) – VSVFPLE —murine IgM Fc
CR2 (SCR1-2) – VSVFPLE —CVF
CR2 (SCR1-2) --- *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester --DAF

CR2 (SCR1-2) --- *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —human CD59

CR2 (SCR1-2) —-*m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester --MCP

CR2 (SCR1-2) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —CR1

CR2 (SCR1-2) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester --Crry

CR2 (SCR1-2) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —mouse CD59

CR2 (SCR1-2) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —human IgG1 Fc

CR2 (SCR1-2) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —human IgM Fc

CR2 (SCR1-2) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —murine IgG3 Fc

CR2 (SCR1-2) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —murine IgM Fc

CR2 (SCR1-2) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —CVF

CR2 (SCR1-2) – bismaleimidohexane --DAF

CR2 (SCR1-2) – bismaleimidohexane —human CD59

CR2 (SCR1-2) – bismaleimidohexane --MCP

CR2 (SCR1-2) – bismaleimidohexane —CR1

CR2 (SCR1-2) – bismaleimidohexane --Crry

CR2 (SCR1-2) – bismaleimidohexane —mouse CD59

CR2 (SCR1-2) – bismaleimidohexane —human IgG1 Fc

CR2 (SCR1-2) – bismaleimidohexane —human IgM Fc

CR2 (SCR1-2) – bismaleimidohexane —murine IgG3 Fc

CR2 (SCR1-2) – bismaleimidohexane —murine IgM Fc

CR2 (SCR1-2) – bismaleimidohexane —CVF

CR2 (SCR1-3) – $(Gly_4Ser)_3$--DAF

CR2 (SCR1-3) – $(Gly_4Ser)_3$—human CD59

CR2 (SCR1-3) – $(Gly_4Ser)_3$--MCP

CR2 (SCR1-3) – $(Gly_4Ser)_3$—CR1
CR2 (SCR1-3) – $(Gly_4Ser)_3$--Crry
CR2 (SCR1-3) – $(Gly_4Ser)_3$—mouse CD59
CR2 (SCR1-3) – $(Gly_4Ser)_3$—human IgG1 Fc
CR2 (SCR1-3) – $(Gly_4Ser)_3$—human IgM Fc
CR2 (SCR1-3) – $(Gly_4Ser)_3$—murine IgG3 Fc
CR2 (SCR1-3) – $(Gly_4Ser)_3$—murine IgM Fc
CR2 (SCR1-3) – $(Gly_4Ser)_3$--CVF
CR2 (SCR1-3) – $(Gly_3Ser)_4$--DAF
CR2 (SCR1-3) – $(Gly_3Ser)_4$—human CD59
CR2 (SCR1-3) – $(Gly_3Ser)_4$--MCP
CR2 (SCR1-3) – $(Gly_3Ser)_4$—CR1
CR2 (SCR1-3) – $(Gly_3Ser)_4$--Crry
CR2 (SCR1-3) – $(Gly_3Ser)_4$—mouse CD59
CR2 (SCR1-3) – $(Gly_3Ser)_4$—human IgG1 Fc
CR2 (SCR1-3) – $(Gly_3Ser)_4$—human IgM Fc
CR2 (SCR1-3) – $(Gly_3Ser)_4$—murine IgG3 Fc
CR2 (SCR1-3) – $(Gly_3Ser)_4$—murine IgM Fc
CR2 (SCR1-3) – $(Gly_3Ser)_4$—CVF
CR2 (SCR1-3) – $(Gly_4Ser)_3$—DAF (SCRs 2-4)
CR2 (SCR1-3) – $(Gly_3Ser)_4$—DAF (SCRs 2-4)
CR2 (SCR1-3) – $(Gly_4Ser)_3$—CR1 (LP--SCR1-4—SCR8-11—SCR15-18)
CR2 (SCR1-3) – $(Gly_4Ser)_3$—Crry (5 N-terminal SCRs)
CR2 (SCR1-3) – VSVFPLE--DAF
CR2 (SCR1-3) – VSVFPLE —human CD59
CR2 (SCR1-3) – VSVFPLE --MCP
CR2 (SCR1-3) – VSVFPLE —CR1
CR2 (SCR1-3) – VSVFPLE --Crry
CR2 (SCR1-3) – VSVFPLE —mouse CD59
CR2 (SCR1-3) – VSVFPLE —human IgG1 Fc

CR2 (SCR1-3) – VSVFPLE —human IgM Fc

CR2 (SCR1-3) – VSVFPLE —murine IgG3 Fc

CR2 (SCR1-3) – VSVFPLE —murine IgM Fc

CR2 (SCR1-3) – VSVFPLE —CVF

CR2 (SCR1-3) --- *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester --DAF

CR2 (SCR1-3) --- *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —human CD59

CR2 (SCR1-3) —-*m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester --MCP

CR2 (SCR1-3) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —CR1

CR2 (SCR1-3) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester --Crry

CR2 (SCR1-3) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —mouse CD59

CR2 (SCR1-3) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —human IgG1 Fc

CR2 (SCR1-3) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —human IgM Fc

CR2 (SCR1-3) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —murine IgG3 Fc

CR2 (SCR1-3) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —murine IgM Fc

CR2 (SCR1-3) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —CVF

CR2 (SCR1-3) – bismaleimidohexane --DAF

CR2 (SCR1-3) – bismaleimidohexane —human CD59

CR2 (SCR1-3) – bismaleimidohexane --MCP

CR2 (SCR1-3) – bismaleimidohexane —CR1

CR2 (SCR1-3) – bismaleimidohexane --Crry

CR2 (SCR1-3) – bismaleimidohexane —mouse CD59

CR2 (SCR1-3) – bismaleimidohexane —human IgG1 Fc

CR2 (SCR1-3) – bismaleimidohexane —human IgM Fc

CR2 (SCR1-3) – bismaleimidohexane —murine IgG3 Fc

CR2 (SCR1-3) – bismaleimidohexane —murine IgM Fc

CR2 (SCR1-3) – bismaleimidohexane —CVF

CR2 (SCR1-4) – $(Gly_4Ser)_3$--DAF

CR2 (SCR1-4) – $(Gly_4Ser)_3$—human CD59

CR2 (SCR1-4) – $(Gly_4Ser)_3$--MCP

CR2 (SCR1-4) – $(Gly_4Ser)_3$—CR1

CR2 (SCR1-4) – $(Gly_4Ser)_3$--Crry

CR2 (SCR1-4) – $(Gly_4Ser)_3$—mouse CD59

CR2 (SCR1-4) – $(Gly_4Ser)_3$—human IgG1 Fc

CR2 (SCR1-4) – $(Gly_4Ser)_3$—human IgM Fc

CR2 (SCR1-4) – $(Gly_4Ser)_3$—murine IgG3 Fc

CR2 (SCR1-4) – $(Gly_4Ser)_3$—murine IgM Fc

CR2 (SCR1-4) – $(Gly_4Ser)_3$--CVF

CR2 (SCR1-4) – $(Gly_3Ser)_4$--DAF

CR2 (SCR1-4) – $(Gly_3Ser)_4$—human CD59

CR2 (SCR1-4) – $(Gly_3Ser)_4$--MCP

CR2 (SCR1-4) – $(Gly_3Ser)_4$—CR1

CR2 (SCR1-4) – $(Gly_3Ser)_4$--Crry

CR2 (SCR1-4) – $(Gly_3Ser)_4$—mouse CD59

CR2 (SCR1-4) – $(Gly_3Ser)_4$—human IgG1 Fc

CR2 (SCR1-4) – $(Gly_3Ser)_4$—human IgM Fc

CR2 (SCR1-4) – $(Gly_3Ser)_4$—murine IgG3 Fc

CR2 (SCR1-4) – $(Gly_3Ser)_4$—murine IgM Fc

CR2 (SCR1-4) – $(Gly_3Ser)_4$—CVF

CR2 (SCR1-4) – $(Gly_4Ser)_3$—DAF (SCRs 2-4)

CR2 (SCR1-4) – $(Gly_3Ser)_4$—DAF (SCRs 2-4)

CR2 (SCR1-4) – $(Gly_4Ser)_3$—CR1 (LP--SCR1-4—SCR8-11—SCR15-18)

CR2 (SCR1-4) – $(Gly_4Ser)_3$—Crry (5 N-terminal SCRs)

CR2 (SCR1-4) – VSVFPLE--DAF

CR2 (SCR1-4) – VSVFPLE —human CD59

CR2 (SCR1-4) – VSVFPLE --MCP

CR2 (SCR1-4) – VSVFPLE —CR1

CR2 (SCR1-4) – VSVFPLE --Crry

CR2 (SCR1-4) – VSVFPLE —mouse CD59

CR2 (SCR1-4) – VSVFPLE —human IgG1 Fc

CR2 (SCR1-4) – VSVFPLE —human IgM Fc

CR2 (SCR1-4) – VSVFPLE —murine IgG3 Fc

CR2 (SCR1-4) – VSVFPLE —murine IgM Fc

CR2 (SCR1-4) – VSVFPLE —CVF

CR2 (SCR1-4) --- *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester --DAF

CR2 (SCR1-4) --- *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —human CD59

CR2 (SCR1-4) —-*m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester --MCP

CR2 (SCR1-4) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —CR1

CR2 (SCR1-4) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester --Crry

CR2 (SCR1-4) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —mouse CD59

CR2 (SCR1-4) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —human IgG1 Fc

CR2 (SCR1-4) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —human IgM Fc

CR2 (SCR1-4) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —murine IgG3 Fc

CR2 (SCR1-4) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —murine IgM Fc

CR2 (SCR1-4) — *m*-Maleimidobenzoyl-*N*-hydoxysuccinimide ester —CVF

CR2 (SCR1-4) – bismaleimidohexane --DAF

CR2 (SCR1-4) – bismaleimidohexane —human CD59

CR2 (SCR1-4) – bismaleimidohexane --MCP

CR2 (SCR1-4) – bismaleimidohexane —CR1

CR2 (SCR1-4) – bismaleimidohexane --Crry

CR2 (SCR1-4) – bismaleimidohexane —mouse CD59

CR2 (SCR1-4) – bismaleimidohexane —human IgG1 Fc

CR2 (SCR1-4) – bismaleimidohexane —human IgM Fc

CR2 (SCR1-4) – bismaleimidohexane —murine IgG3 Fc

CR2 (SCR1-4) – bismaleimidohexane —murine IgM Fc

CR2 (SCR1-4) – bismaleimidohexane —CVF

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H. Sequences

1. DAF

Nucleotide Sequence corresponds to SEQ ID NO: 1
Amino Acid Sequence corresponds to SEQ ID NO: 2

2. CD59

Nucleotide Sequence corresponds to SEQ ID NO: 3
Amino Acid Sequence corresponds to SEQ ID NO: 4

3. CR2-DAF

Nucleotide Sequence corresponds to SEQ ID NO: 5
Amino Acid Sequence corresponds to SEQ ID NO: 6

4. CR2-human CD59

Nucleotide Sequence corresponds to SEQ ID NO: 7
Amino Acid Sequence corresponds to SEQ ID NO: 8

5. DAF-CR2

Nucleotide Sequence corresponds to SEQ ID NO: 9
Amino Acid Sequence corresponds to SEQ ID NO: 10

6. human CD59-CR2

Nucleotide Sequence corresponds to SEQ ID NO: 11
Amino Acid Sequence corresponds to SEQ ID NO: 12

7. CR1

Nucleotide Sequence corresponds to SEQ ID NO: 13
Amino Acid Sequence corresponds to SEQ ID NO: 14

8. MCP

Nucleotide Sequence corresponds to SEQ ID NO: 15
Amino Acid Sequence corresponds to SEQ ID NO: 16

9. Mouse Crry

Amino Acid Sequence corresponds to SEQ ID NO: 17

10. human IgG1 Fc

Amino Acid Sequence corresponds to SEQ ID NO: 18

11. human IgM Fc

Amino Acid Sequence corresponds to SEQ ID NO: 19

12. CR2-human IgG1 Fc

Nucleotide Sequence corresponds to SEQ ID NO: 20
Amino Acid Sequence corresponds to SEQ ID NO: 21

13. mouse IgG3 Fc

Amino Acid Sequence corresponds to SEQ ID NO: 22

14. Cobra venom factor

Nucleotide Sequence corresponds to SEQ ID NO: 23
Amino Acid Sequence corresponds to SEQ ID NO: 24

15. Human CR2

Nucleotide Sequence corresponds to SEQ ID NO: 25
Amino Acid Sequence corresponds to SEQ ID NO: 26

16. Mouse CR2

Nucleotide Sequence corresponds to SEQ ID NO: 27
Amino Acid Sequence corresponds to SEQ ID NO: 28

17. Human CR2

Amino Acid Sequence corresponds to SEQ ID NO: 29